Food Research

IN THIS ISSUE

Growth, Reproduction, Survival and Histopathology of Rats Fed Beef Irradiated With Electrons

Toxicological Determination of Suitability of Food Additives

The Effect of Sweating on Keeping Quality of Shell Eggs

Effect of Reproduction in Bacterial Load Due to Previous Exposure to Heat During Thermal Process on Lethal Rates and Lethality

Heat Transfer Into Open Metal Thermoresistometer Cups

Reduced and Total Ascorbic Acid Values of Thirty-four Foods

The Respiratory Enzymes of Meat. I. Identification of the Active Enzymes

The Comparative Effect of Ionizing Radiations and Heat Upon the Starch Containing Cells of the Potato Tuber

Denaturation of Proteins of Egg White and of Fish and Its Relation to the Liberation of Sulfhydryl Groups on Frozen Storage

Effect of Freezing and Freezer Storage on Cake Quality. I. Baked Spice Cakes and Cakes Baked from Frozen Batters

Effect of Freezing and Freezer Storage on Cake Quality. II. Variation in Batter Treatment Before and After Freezer Storage

A Chemical Study of the Progressive Development of Off-Flavor in Frozen Raw Vegetables

Notes and Letters—Descriptive Terms and Grading Systems

VOLUME 20, No. 3 **MAY-JUNE, 1955**

An Official Publication of

The Institute of Food Technologists

Food Research

An Official Publication of
The Institute of Food Technologists

Volume 20 MAY-JUNE, 1955 Number 3

EDITORIAL BOARD

MARTIN S. PETERSON, *Editor-in-Chief*
(Editorial Office: 11606 South Bell Avenue,
Chicago 43, Illinois)

J. GRANT ARMSTRONG
J. S. BLAIR
KENNETH W. BRIGHTON
FRANK L. GUNDERSON
R. R. LEGAULT
ANDREA MACKEY
JOHN R. MATCHETT
LOUIS B. ROCKLAND
H. L. A. TARR
ROBERT G. TISCHER
JAMES RICHARD VICKERY
O. B. WILLIAMS

FRED W. TANNER, *Editor Emeritus*
Z. I. KERTESZ, *Consulting Editor*

CHARLES S. LAWRENCE, *Business Manager*
176 West Adams Street
Chicago 3, Illinois

Copyright, 1955
By the Institute of Food Technologists
Printed in U. S. A.

Published by the Institute of Food Technologists six times a year. Printed by The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois. Entered as second-class matter at the Post Office at Champaign, Illinois, under the act of March 3, 1879. Accepted for mailing at special rate of postage provided for in amended Section 34.40(e), pursuant to the provisions of Public Law 233, approved October 8, 1951.

INSIDE SCIENCE

The Vital Story of VITAMIN C

by Science Writer

What does vitamin C do for human beings?

Medical research shows that it is essential in maintaining healthy bony tissue and for the formation of collagen which is the main supportive protein of skin, tendon, bone, cartilage and connective tissue. It is needed for vascular function and tissue respiration and in promoting the healing of wounds. In lactating women it has great importance in providing the infant with a sufficient amount of ascorbic acid to prevent scurvy, a disease to which very young babies are susceptible.

Much evidence is available in medical reports to show that a lack of this vital nutritive element can result in impaired health. And in those cases of frank scurvy which still occur, ascorbic acid is the specific medication.

History. The history of vitamin C is fascinating. Scurvy was once the most prevalent disease in Europe. In the 18th Century the antiscorbutic value of fresh vegetables and citrus fruits was recognized.

Isolation. It was not until 1918-25 that almost pure ascorbic acid was concentrated from the lemon by Zilva and its important properties and molecular composition established. In 1927 he concluded that this so-called "reducing factor" and the antiscorbutic principle were closely related. In 1928, Szent-Györgyi, who started investigations in 1921, isolated a strong reducing compound from adrenal glands, oranges, cabbage, terming it "hexuronic acid."

Chemistry. The "reducing factor," "hexuronic acid," and vitamin C were all identified as the same in 1932 by various groups of workers. This was one year after C. G. King established the chemical identification of vitamin C.

Synthesis. In 1933 Reichstein and his colleagues in Switzerland successfully synthesized ascorbic acid. Almost simultaneously in England Haworth and associates accomplished the same. The Reichstein synthesis formed the basis for large scale commercial production by the world-famous firm of Hoffmann-La Roche.

Production. Following these discoveries came the establishment of manufacturing operations so that today daily production by the tons is the rule. The figures are really staggering. The amount produced weekly in the U. S. A. averages over 15 tons, equivalent to that contained in over 200 million oranges. It is remarkable that ascorbic acid can be produced synthetically and sold for only about $16. per kilogram. One kilo is sufficient to supply 92 people for one year with the minimum daily requirements set by the U. S. Food and Drug Administration.

Vitamin C made by the Roche process is identical chemically and in biological activity with Nature's own product. Because vitamins can be manufactured at a much lower cost than if they were extracted from natural sources they are widely used in dietary supplements.

Ascorbic acid is present in many foods. Nature supplies some with lavish amounts and stints on others. In fact, wide variations* have been found in the vitamin C content in varieties of the same fruit.

Deficiencies. Many surveys have shown widespread vitamin C deficiencies in the population. Physicians may call these subclinical, but they are deficiencies nevertheless.

Because of these situations—natural variation and widespread deficiencies—diet experts believe that it is desirable and in the public interest to standardize the vitamin C content of processed fruit and vegetable juices by the addition of enough pure crystalline ascorbic acid to make them consistently dependable sources of this necessary food element. Some of these juices are: tomato, grape, orange, grapefruit, pineapple.

Requirements. The U. S. Food & Drug Administration has set 30 milligrams as the adult minimum daily requirement of ascorbic acid. This daily intake is just about sufficient to prevent recognizable signs of vitamin C deficiencies although a subclinical or an unrecognized deficiency may exist. In view of this, the National Research Council has established 75 milligrams as the recommended daily male adult allowance and 70 mg. daily for female adults, which levels are supported by many controlled nutrition studies. However, the N. R. C. recommends 90 mg. daily for boys between 13 and 15, 100 mg. daily for boys between 16 and 20, and 150 mg. daily for lactating women.

This article is published in the interests of pharmaceutical manufacturers and food processors who make their good products better with essential, health-giving vitamin C. Reprints are freely available. Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

*The table shows minimum and maximum levels of ascorbic acid in commercially canned juices. All figures are in milligrams per 100 grams of juice. Data from U. S. Department of Agriculture.

	Min.	*Max.*
Apple juice	0.2	3.6
Grape juice	0.0	4.7
Grapefruit juice	10.0	49.0
Orange juice	9.7	70.0
Pineapple juice	5.4	18.0
Tomato juice	2.5	32.0

Volume 20 MAY-JUNE, 1955 Number 3

CONTENTS

GROWTH, REPRODUCTION, SURVIVAL AND HISTOPATHOLOGY OF RATS FED BEEF IRRADIATED WITH ELECTRONS. C. E. Poling, W. D. Warner, F. R. Humburg, E. F. Reber, W. M. Urbain, and E. E. Rice 193

TOXICOLOGICAL DETERMINATION OF SUITABILITY OF FOOD ADDITIVES. Harry J. Deuel, Jr. 215

THE EFFECT OF SWEATING ON KEEPING QUALITY OF SHELL EGGS. I. L. Williams and F. D. Yung 221

EFFECT OF REPRODUCTION IN BACTERIAL LOAD DUE TO PREVIOUS EXPOSURE TO HEAT DURING THERMAL PROCESS ON LETHAL RATES AND LETHALITY. H. Hurwicz and R. G. Tischer 226

HEAT TRANSFER INTO OPEN METAL THERMORESISTOMETER CUPS. I. J. Pflug and W. B. Esselen 237

REDUCED AND TOTAL ASCORBIC ACID VALUES OF THIRTY-FOUR FOODS. Katherine H. Fisher and Mary L. Dodds 247

THE RESPIRATORY ENZYMES OF MEAT. I. IDENTIFICATION OF THE ACTIVE ENZYMES. Norman H. Grant 250

THE COMPARATIVE EFFECT OF IONIZING RADIATIONS AND HEAT UPON THE STARCH CONTAINING CELLS OF THE POTATO TUBER. Edith A. Roberts and Bernard E. Proctor 254

DENATURATION OF PROTEINS OF EGG WHITE AND OF FISH AND ITS RELATION TO THE LIBERATION OF SULFHYDRYL GROUPS ON FROZEN STORAGE. Saeed Ahmad Husaini and Frithiof Alm 264

EFFECT OF FREEZING AND FREEZER STORAGE ON CAKE QUALITY. I. BAKED SPICE CAKES AND CAKES BAKED FROM FROZEN BATTERS. Doris M. Skarha and Frances O. Van Duyne 273

EFFECT OF FREEZING AND FREEZER STORAGE ON CAKE QUALITY. II. VARIATION IN BATTER TREATMENT BEFORE AND AFTER FREEZER STORAGE. Doris M. Skarha and Frances O. Van Duyne 282

A CHEMICAL STUDY OF THE PROGRESSIVE DEVELOPMENT OF OFF-FLAVOR IN FROZEN RAW VEGETABLES. Frank A. Lee, A. C. Wagenknecht, and J. C. Hening . . 289

NOTES AND LETTERS—DESCRIPTIVE TERMS AND GRADING SYSTEMS 298

SUBSCRIPTION RATES

To members of the Institute of Food Technologists, $7.50 per volume.

Non-members: Domestic, $8.50 per volume; foreign, $10.00 per volume.

Subscriptions and renewals are entered to begin with the first issue of the current volume.

Single copies of current volume: Domestic, $1.00; foreign, $1.25.

CORRESPONDENCE

Editorial: MARTIN S. PETERSON, Editor-in-Chief, 11606 South Bell Ave., Chicago 43, Ill.

Non-Member Subscriptions, Back Volumes, Single Copies, and Reprints: FOOD RESEARCH, 119-123 W. Park Ave., Champaign, Ill.

Members Subscriptions: THE INSTITUTE OF FOOD TECHNOLOGISTS, 176 W. Adams St., Chicago 3, Ill.

Advertising: Charles S. Lawrence, Business Manager, 176 W. Adams St., Chicago, Ill.

CLAIMS

Claims for copies lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

NOTE

The Institute of Food Technologists assumes no responsibility for statements in articles appearing in FOOD RESEARCH. The opinions expressed do not necessarily reflect policies of the Institute of Food Technologists.

Manuscripts for FOOD RESEARCH should conform to the style used in the journal and be submitted in duplicate to the Editor-in-Chief. Authors of papers intended for FOOD RESEARCH should request from the Editorial Office a copy of the booklet "Information and suggestions for authors." FOOD RESEARCH reserves the privilege of editing manuscripts to make them conform with the adopted style of the journal or returning them to the author for revision. Changes made by the Editorial Office may be reviewed by authors when they examine proof.

Official

Directory of Members

1952 - 1953

The Institute of Food Technologists

For the first time in over 3 years, the Institute announces publication of a complete membership directory, listing more than 3,300 active members, together with their mailing addresses, business or professional affiliations, and titles.

The new Directory offers you a quick, authoritative reference source of personnel information and business and professional addresses, conveniently arranged for your daily use. Order your personal copy now.

Price to Members	**$2.00**
Non-Members	**$5.00**

Published for

The Institute of Food Technologists

Send your order direct to

THE GARRARD PRESS

Champaign **Illinois**

GROWTH, REPRODUCTION, SURVIVAL AND HISTOPATHOLOGY OF RATS FED BEEF IRRADIATED WITH ELECTRONS

C. E. POLING, W. D. WARNER, F. R. HUMBURG
E. F. REBER[a], W. M. URBAIN, AND E. E. RICE
Research Laboratories, Swift & Company, Chicago, Illinois

(Manuscript received February 25, 1955)

The retarding or control of microbial growth in foods is commonly accomplished by refrigeration, cooking, canning, salting, or drying. For many purposes these methods are satisfactory, but for others they leave much to be desired. Recent demonstrations that ionizing radiations will destroy or at least prevent the multiplication of bacteria have stimulated interest in the use of this form of radiant energy in the preservation of foods.

Beams of relatively fast-moving electrons, more commonly known as cathode or beta rays, are effective for this purpose. In accomplishing this bactericidal action, the rays cause no significant temperature rise and leave no residual radioactvity. The procedure may be considered as a *cold* sterilization process. Also, beta rays penetrate materials and consequently, unlike ultraviolet light, exert bactericidal action throughout the food.

The use of electron beams in treating food for the purpose of controlling the microbial growth offers many possibilities. Most of these center about the fact that the process induces no significant rise in temperature. Thus one may obtain, for example, a piece of raw meat which is completely sterile. Many, if not all, of the enzymes may remain active. Investigations have shown that under certain conditions the treatment of meat with electrons produces desirable results.

This report describes experiments made with rats to determine the effect of beta irradiation upon the wholesomeness of beef as a food. It includes methods of irradiation, observations of the relative growth, food efficiencies, reproductive capacities, adult survivals, and histopathological abnormalities of rats fed diets containing irradiated or non-irradiated beef.

PROCEDURE

Irradiation of beef. A definite amount of radiant energy is required to inactivate bacteria. It is generally considered that approximately two million roentgen-equivalents-physical (rep) *(18)* are a sterilizing dose sufficient to destroy even the most resistant forms of food spoilage bacteria, including spores. For this experiment this level of treatment was chosen since it approximated the most likely maximum commercial application dose. Further, as will be seen below, provision of a minimum dose of 2×10^6 rep inherently included some overdosage, making the average dose higher and thereby allowing a safety factor not much different from that to be expected in commercial use. Levels of treatment above 2×10^6 rep were believed to be unrealistic and possibly misleading for normal meat products.

Due to the absorption characteristics of cathode rays and to the distribution patterns of the generators used *(7)* it was not possible to obtain a uniform dose throughout

[a] Present address: College of Veterinary Medicine, University of Illinois, Urbana, Illinois.

the portion being irradiated. The non-uniformity was minimized by controlling the size and shape of the portion being treated and by irradiating both top and bottom surfaces. In order to maintain definite physical dimensions, all samples were kept frozen during irradiation.

In these experiments the meat was irradiated so that no portion received less than approximately 2×10^6 rep. This caused the average to be somewhat higher depending on the equipment used. Approximately half of the irradiated meat used in this experiment was treated with a Capacitron cathode ray source and the rest was treated with a resonant transformer type source.

The Capacitron *(10)* periodically emitted micro-second bursts of electrons with energies of about two million volts. Calibration of the beam output energy was made calorimetrically. These electrons form a circular pattern of greatest intensity at the center. In order to permit uniform treatment the meat was formed into concentric rings with a thickness of 1.27 cm. The first of these was a disc 14 cm. in diameter; succeeding rings had inside and outside diameters as follows: 15.3 to 20.3 cm.; 21.6 to 26.7 cm.; and 27.9 to 35.6 cm. Each of these was held in the electron pattern until a calculated number of bursts had been applied. The dose within any ring varied from a minimum of 1.9×10^6 rep to a maximum of 6.3×10^6 rep, and averaged 3.7×10^6 rep on the basis of 1 rep = 93 ergs per g.

The General Electric resonant transformer type of source was used to treat the balance of the meat. This unit emits a relatively continuous beam of electrons at an accelerating voltage of 800,000 peak volts. The calibration of the beam output energy was made with an ionization chamber *(13)*.

Portions of frozen meat ($6.35 \times 6.35 \times 0.32$ cm.) were passed continuously on a conveyor system through the electron beam at a distance of 10 cm. from the tube window. The conveyor speeds and beam current were adjusted so that the dose was a maximum of 3.0×10^6 rep and a minimum of 2.2×10^6 rep. The average was 2.7×10^6 rep on the same basis as above.

Except for the differences noted for the range of dose maxima and averages, the samples treated with either the Capacitron or the resonant transformer received identical treatments, and no differences between the meat irradiated by the 2 units are assumed. The variation in dose maximum and average is not regarded as significant, the essential identity of the minimum dose being the governing consideration.

Animal feeding experiment. Before beginning the long-term animal feeding experiment, feeding tests had been conducted to provide preliminary information considered desirable. The results of these tests indicated: (a) that rats would thrive for several weeks on diets in which meat supplied as much as 89% of the solids, (b) that in such diets the type of carbohydrate was not critical, (c) that rats would eat readily diets containing high levels of irradiated beef, and (d) that irradiation with 5×10^6 rep decreased the nutritional quality for growth of beef protein only slightly and less than retorting according to a regular commercial processing schedule for canning.

For the long-term feeding experiment, weanling rats of the Holtzman strain were procured and conditioned for 3 days on a stock diet. These were divided into two groups of 80 rats each (40 of each sex) with comparable average starting weights and distribution of weights. The rats were caged individually in $17.8 \times 25.4 \times 17.8$ cm. cages except during reproduction and housed in an air-conditioned animal room maintained at $27° \pm 1°$ and 40-50% relative humidity. Those animals which became very large (over 500 g.) were transferred to $41.9 \times 25.4 \times 17.8$ cm. cages.

Diets intended to provide all known nutritional requirements of the rat were formulated for both groups. Excluding the water contained in the meat, the percentage formulation of the diets was: beef solids, 45; corn starch, 43; salt mixture,[b] 4; liver concentrate (Wilson's N.F.), 2; Ruffex,[c] 2; vitamin A & D oil (2250 U.S.P. units of A, 300 I.C.U. of D_3), 1; wheat germ oil, 1; yeast (Annheuser-Busch Type 300), 1;

[b] The salt mixture was a phosphorus free, high calcium modification of that of Jones and Foster *(11)*. This modification was considered desirable because of the large contribution of available phosphorus by the beef hamburger. In the mixture used 216.0 g. of $CaCO_3$ and 600.6 g. of $KHCO_3$ replaced 816.6 g. of KH_2PO_4.

[c] Ruffex is ground rice hulls processed to serve as non-nutritive roughage.

TABLE 1

Proximate analyses of diets

	Control		Experimental	
	As fed	Dry (calc.)	As fed	Dry (calc.)
	%	%	%	%
Moisture	42.0	.....	41.6	.....
Solids	58.0	100.0	58.4	100.0
Protein (N × 6.25)	11.6	20.0	12.1	20.7
Fat	17.2	29.6	16.7	28.6
Ash	2.68	4.62	2.80	4.79
Calcium	0.48	0.83	0.47	0.80
Phosphorus	0.31	0.53	0.32	0.55
Ca/P	1.55	.....	1.46	.....

[1] Averages of seven separate preparations of each diet.

and orthophosphoric acid (85% H_3PO_4), 1. During the 11th week and for the duration of the experiment 1 mg. per cent of 2 methyl naphthoquinone (Menadione) was added to both diets, although there is little, if any, evidence that the rat requires a dietary source of this vitamin.

Diets fed the experimental and control groups were identical except that beef hamburger irradiated as described above was used in the experimental diet while unirradiated hamburger was used in the control diet. The raw beef hamburger used was a commercial product[4] untreated except for grinding, freezing, and storage at −18° until needed for irradiation or diet preparation.

In preparing the diets, all ingredients except the beef were premixed in quantities sufficient for 2 to 3 weeks feeding and stored at 5-10° until used. This premix was then mixed with either raw or irradiated hamburger in the ratio of 112 g. hamburger (equivalent to 45 g. beef solids) to 55 g. premix. Fresh diets were made weekly for the first 10 months; after this they were prepared at intervals of 10 to 12 days. The diets were divided into portions sufficient for one day's feeding, wrapped in heavy wax paper, frozen quickly, and stored at about −18° until used. The diets were thawed just before feeding (with the exception of week-ends and holidays) and were fed daily *ad libitum*.

The beef hamburger comprised about two-thirds of each diet as fed and provided 60-65% of the total calories. The essential similarity of the diets in content of major nutrients is indicated in Table 1 by the average proximate analysis of 7 batches of each diet.

The original two groups of 80 rats each (hereafter referred to as the parent generation) were maintained on their respective diets for 2 years without change in formulation of the diets or any supplementation except the addition of vitamin K, as noted above. During this period, comparisons were made of: growth and adult body size (weekly weight records), food consumption,[5] reproduction, mortality, hematology, tumors, and gross and histopathology on representative animals at 3, 6, 12, 18 and 24 months (end of the experiment). General observations were made on a daily basis, with a critical examination once weekly, of the grossly apparent state of health of each animal. Apparent deviations from a normal state, even if slight, were recorded chronologically for each animal. The data, wherever feasible, were subjected to statistical

[4] Swift & Company's Arrow S brand.

[5] In general complete individual food records were kept meticulously for all animals on a weekly basis by calculating from the weight of wet diet offered the equivalent weight of dry solids and subtracting from this weight the actual weight of dry solids returned uneaten. All uneaten and scattered food was collected (daily except week-ends and holidays) in individual containers for each animal and dried weekly at about 116° before weighing. Although food records were kept during the reproduction phase of the experiment, it was impractical to keep individual records for each animal at this time because of the necessity for multiple occupancy caging.

analysis and throughout the paper differences between groups have been described with the conventional usage of significant ($P < 0.05$) and highly significant ($P < 0.01$).

The original animals were mated for the production of first and second litters of first generation offspring. The first litter weanlings were discarded, and representative second litter weanlings were saved for growth and production of first and second litters of second generation offspring. This same procedure was repeated with the second generation to obtain first and second litters of third generation offspring. The first litter third generation weanlings were discarded and the second litter weanlings were sacrificed for histopathological examination of representative animals.

Although the plan outlined above was followed in general, the results as they were obtained necessitated some modification in the details of the procedure which will be explained later in reporting the results.

Growth and Adult Weights

Figure 1 shows that the growth of animals fed both control and experimental diets was exceptionally good in the 3 generations which were carried through the 9-week initial growth period. The similarity in the growth response of all rats fed the control and experimental diets is evident, as is the fact that there was no cumulative decline in growth for each succeeding generation. The experimental males gained slightly less in each generation than the control males; the difference in gains was not significant ($P < 0.07$) in the parent generation and the second generation but was significant in the first generation. For the females, growth on the experimental diet did not differ significantly from that on the control in any generation.

The parent generation rats were continued on the experiment to complete a 2-year period. The first and second generations were continued long enough to permit each female a reasonable opportunity to bear 2 litters (30 weeks for the males and 35 weeks for the females). During the 30-week period the male animals, in particular, developed to a very large size for both groups in all generations. The parent generation males, especially the controls, eventually became exceptionally large during the 2-year period and probably could best be described as obese (four actually attained maximum weights over 800 g.; one of these was over 900). The parent generation females, although well nourished, did not tend to store fat to an excessive extent until after the second litters were weaned when some of them likewise became quite obese.

The data in Table 2 demonstrate that the experimental males were very large animals although their adult weights progressively fell below those of the control males in all generations. The differences were larger and were significant after 14 weeks in the parent and first generation, but never became significant for the second generation. The largest difference occurred in the parent generation and was highly significant after 30 weeks. Although the difference continued to increase progressively through 78 weeks and remained significant for the parent generation, the survivors after 104 weeks (end of the experiment) showed a much smaller difference, which was not significant.

The adult weights of the females in each group were difficult to compare during the period 10 to 35 weeks because of the effects of pregnancy and lactation upon weight. However, the parent generation experimental females were not significantly smaller than the control females until the 104th week. The experimental females were not significantly smaller than the control females in either the first or second generations when discarded at 35 weeks.

When comparing one generation with the following one, the average weights at 14 and 30 weeks on experiment for both the first and second generation animals usually equaled and often exceeded those of the parent generation. This was true for both sexes within both groups. If the assumption is made that a direct comparison of one generation with another is valid, then the following comparisons can be made, the first group weighing more than the second:

1. Differences highly significant at times indicated.
   a. Control first generation males: control parent generation males (30 weeks).
   b. Experimental second generation males: experimental parent generation males (14 weeks).

c. Experimental second generation males: experimental first generation males (14 weeks).

2. Differences significant at times indicated.
   a. Control second generation males: control parent generation males (14 weeks).
   b. Experimental first generation males: experimental parent generation males (30 weeks).
   c. Experimental second generation males: experimental parent generation males (30 weeks).
   d. Control first generation females: control parent generation females (14 weeks).
   e. Control first generation females: control second generation females (14 weeks).

In connection with the reproduction phase of the experiment, about one half of the animals of each sex in each dietary group of the first generation were given an oral supplement of 10 mg. DL alpha tocopherol five times weekly beginning with the

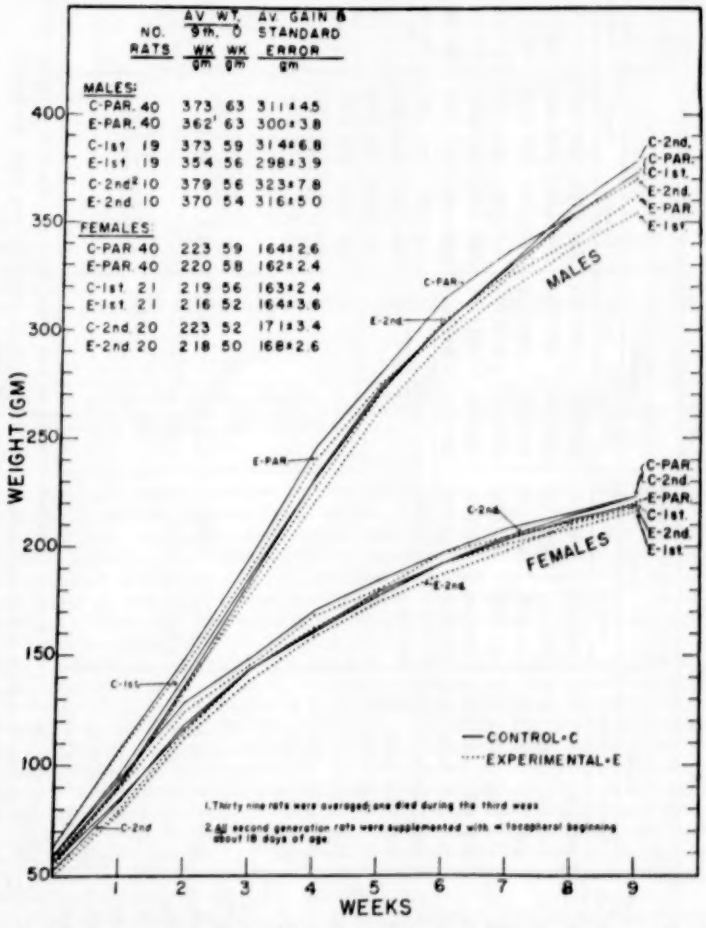

NO. RATS
AV. WT. 9th. WK gm
AV. WT. 0 WK gm
AV. GAIN & STANDARD ERROR gm
MALES:
C-PAR 40 373 63 311±4.5
E-PAR 40 362[1] 63 300±3.8
C-1st 19 373 59 314±6.8
E-1st 19 354 56 298±3.9
C-2nd[2] 10 379 56 323±7.8
E-2nd 10 370 54 316±5.0
FEMALES:
C-PAR 40 223 59 164±2.6
E-PAR 40 220 58 162±2.4
C-1st 21 219 56 163±2.4
E-1st 21 216 52 164±3.6
C-2nd 20 223 52 171±3.4
E-2nd 20 218 50 168±2.6
WEIGHT (GM)
WEEKS
MALES
FEMALES
C-PAR
E-2nd
E-PAR
C-1st
C-2nd
C-2nd, C-PAR, C-1st, E-2nd, E-PAR, E-1st
C-PAR, C-2nd, E-PAR, C-1st, E-2nd, E-1st
—CONTROL=C
·····EXPERIMENTAL=E
1. Thirty nine rats were averaged; one died during the third week.
2. All second generation rats were supplemented with α tocopherol beginning about 18 days of age.

Figure 1. Nine week growth. For parent, first and second generation rats fed diets containing raw (control) or irradiated (experimental) beef hamburger.

TABLE 2

Average adult weights of 3 successive generations fed diets containing raw or irradiated beef hamburger[1]

End of Week	Males—Weights in grams						Females—Weights in grams					
	Parent		First[2]		Second[3]		Parent		First[2]		Second[3]	
	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl
10	380[4] (40)	367[4] (39)	386 (19)	366 (19)	395 (10)	384 (10)	224[4] (40)	222[4] (40)	225 (21)	225 (21)	229 (20)	224 (20)
14	419 (40)	401 (39)	432 (19)	398 (19)	447 (10)	431 (10)	256 (40)	254 (40)	281 (21)	256 (21)	259 (20)	247 (20)
18	454 (38)	432 (37)	483 (19)	443 (19)	486 (10)	460 (10)	286 (38)	282 (37)	281 (21)	278 (21)	302 (20)	287 (20)
22	482 (38)	455 (37)	513 (18)	471 (19)	519 (10)	478 (10)	300 (38)	291 (38)	309 (21)	294 (21)	291 (20)	290 (20)
26	501 (38)	461 (37)	548 (19)	501 (19)	522 (10)	497 (10)	305 (38)	293 (38)	307 (21)	307 (21)	325 (20)	312 (20)
30	525 (36)	494 (35)	578 (19)	522 (19)	540 (10)	517 (10)	312 (36)	303 (36)	307 (21)	307 (21)	323 (20)	310 (20)
35	558 (36)	504 (34)	Sacrificed after 30 weeks				311 (36)	301 (36)	319 (21)	314 (21)	325 (20)	311 (20)
52	651 (36)	584 (32)	.....	.....	.....	.....	340 (35)	324 (35)	Sacrificed after 35 weeks			
65	672 (31)	593 (27)	.....	.....	.....	.....	364 (32)	334 (65)	.....	.....	.....	.....
78	714 (25)	593 (21)	.....	.....	.....	.....	387 (32)	365 (25)	.....	.....	.....	.....
91	646 (14)	610 (9)	.....	.....	.....	.....	406 (24)	369 (15)	.....	.....	.....	.....
104	626 (5)	597 (7)	.....	.....	.....	.....	447 (17)	384 (9)	.....	.....	.....	.....
Standard errors: Week:												
14th	± 5.2	± 4.7	±11.9	±9.6	±11.6	±5.9	±4.5	±4.9	±5.5	±5.8	± 6.1	±3.4
30th	± 6.6	± 6.6	±19.2	±9.3	±24.3	±9.1	.....	.....	.....	.....	.....	.....
35th	.....	.....	.....	.....	.....	.....	±4.6	±4.9	±6.1	±6.7	±10.0	±5.9
52nd	±10.9	±12.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
78th	±15.6	±23.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
104th	±30.7	±34.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[1] Numbers in parentheses represent numbers of animals averaged at each point. Decreases represent mortality, sacrifices or animals not weighed for some reason, e.g., whelping.

[2] About ½ of each sex in each group was supplemented orally with tocopherol, beginning 11th week. See Table 3.

[3] All rats were supplemented orally with tocopherol beginning when about 18 days of age.

[4] One day short of 10 weeks.

11th experimental week (the reasons for giving this supplement will be discussed in detail later). Data in Table 3 show that the supplement did not affect significantly the weights of the males or females in either dietary group with the possible exception of the first generation control males. The unsupplemented subgroup of first generation control males did become significantly larger than the supplemented subgroup by 30 weeks. This growth difference possibly resulted from an uneven distribution of the animals, based on body weight, into subgroups. All animals saved for the second generation received the supplement beginning about 18 days of age; the weights of the experimental males and females did not differ significantly from the control animals.

TABLE 3

Adult weights of unsupplemented and dl alpha tocopherol supplemented subgroups of first generation rats fed diets containing raw or irradiated beef hamburger

End of Week	Males—Average weights in grams				Females—Average weights in grams			
	Control		Experimental		Control		Experimental	
	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented
10	389	384	374	358	227	224	225	218
14	440	425	407	390	284	277	260	251
18	492	476	442	445	276	286	276	281
22	533[1]	498	572	471	306	312	293	295
26	566	531	507	495	308	306	306	309
30	596	562	528	516	305	309	310	303
35	.....	.....	.....	.....	317	322	312	316
Av. gain[2] and standard error	207±18.5	178±14.8	154±10.0	158±13.7	90±8.3	98±5.8	87±5.1	98±8.4
No. animals in each subgroup	9	10	9	10	10	11	11	10

[1] Average represents 8 animals; weighing of one animal was omitted inadvertently.
[2] For period beginning with 11th week, when divided into subgroups for supplementation, and extending to 30th or 35th week when discarded.

Food Utilization

The diets were readily accepted by both experimental and control rats, and, for the most part, the amounts consumed were similar as is shown in Table 4. The similarity continued through at least the 43rd week of the experiment. However, for the 57th to 60th weeks period, the food consumption of the experimental males had become significantly less than that of the controls. The decreased food consumption by the experimental males at this point is correlated perhaps with their significantly smaller size at this age (Table 2). The slightly smaller food consumption of the experimental females was not significant.

The efficiency of food utilization for both control and experimental animals also was excellent in all cases. However, in several instances the experimental animals did not utilize the food consumed quite as efficiently as the control animals. The differences (Table 4) were quite small, but they were highly significant for the parent generation males and females and were significant for the first generation males. The second generation males and first generation females showed no significant differences in efficiency of food utilization while second generation experimental females utilized food more efficiently than the control females, the difference being highly significant. In those instances where the food efficiency was less the males showed a different pattern from the females in that the food consumption was similar, but the experimental males gained a little less; for the females the gains were similar but the experimental females ate a little more food and utilized it less efficiently.

A trend toward increased efficiency of food utilization, usually rather pronounced, was observed for each succeeding generation within each dietary group and sex. If again the assumption is made that a direct comparison of one generation with the

TABLE 4

Food solids consumed and efficiency of food utilization[1]

	First 9 weeks		40-43 weeks	57-60 weeks
	Average solids eaten	Food/gain and standard error	Average solids eaten and standard error	Average solids eaten and standard error
	grams		*grams*	*grams*
Males				
Control (P)	831(40)	2.68 ± 0.02	351 ± 5.7(36)	360 ± 8.8(32)
Exptl (P)	831(39)	2.78 ± 0.02	347 ± 6.4(33)	325[2] ± 13.4(28)
Control (1)	801(19)	2.55 ± 0.03	Rats sacrificed	.....
Exptl (1)	796(19)	2.67 ± 0.04	Rats sacrificed	.....
Control (2)	800(10)	2.48 ± 0.04	Rats sacrificed	.....
Exptl (2)	791(7)	2.43 ± 0.05	Rats sacrificed	.....
Females				
Control (P)	599(40)	3.65 ± 0.03	243 ± 4.6(36)	242 ± 6.5(32)
Exptl (P)	614(40)	3.81 ± 0.03	241 ± 4.5(36)	236 ± 6.4(32)
Control (1)	573(21)	3.48 ± 0.04	Rats sacrificed	.....
Exptl (1)	580(21)	3.53 ± 0.07	Rats sacrificed	.....
Control (2)	583(20)	3.43 ± 0.05	Rats sacrificed	.....
Exptl (2)	546(20)	3.24 ± 0.04	Rats sacrificed	.....

[1] Numbers in parentheses refer to numbers of animals; letters in parentheses refer to generation —(P) = parent, (1) = first, (2) = second generation.
[2] Excluding one animal which ate only 50 g. of food solids.

succeeding one is valid, then the increased efficiency of food utilization would be highly significant in every comparison within dietary groups and sexes except those made between (a) the control males of the first and second generations, and (b) the control females of the first and second generations. The increases in these instances would not be significant, although approaching significance for the males ($P < 0.13$).

These and previous comparisons between generations have been made to emphasize the trend toward better rather than poorer growth and food utilization with each succeeding generation.

Such comparisons demonstrate the overall nutritional adequacy of the diets as well as the absence of a cumulative trend toward poorer performance which might have resulted from cumulative effects of unwholesome substance(s), if present.

Reproduction

The females of each group of the parent generation were mated with males receiving the same diet, at the beginning of the 11th week. One-fourth of the females of each dietary group of the parent generation was mated with males receiving the same diet at the beginning of the 11th week; another fourth was mated two weeks later, etc., until all females had been mated. Each female was caged with a male for one week. At the end of the week a different male was provided. A third male was used for a third week. The females were then isolated for 2 weeks. If pregnancy did not become evident, the cycle was then repeated with additional males. Gravid females were transferred to $41.9 \times 25.4 \times 17.8$ cm. fine mesh cages which were supplied with tissue paper for nesting and examined daily for birth of litters or resorptions. The number of pups counted when the litter was first found was recorded as *pups born*.

Parent generation. The fertility of both control and experimental animals of the parent generation was good (Table 5). The same numbers of first litters were produced. In the second litters there may have been an indication that the fertility of the experimental rats was slightly less than that of the controls. A slightly higher percentage of the experimental females were either difficult breeders or sterile (5.4% for the control compared with 11.4% for the experimental females).

TABLE 5

Summary of reproduction

		Parent generation		First generation				Second generation		Summary
		None supplemented		Unsupplemented		Supplemented		All supplemented		(All generations)
		First litters	Second litters	First litters	Second litters	First litters	Second litters	First litters	Second litters	
Fertility										
Males[1] siring one or more litters	Control	35/40		8/9		7/10		10/10		30/69 = 87%
	Exptl	30/39		4/9		6/10		10/10		50/68 = 74%
Females whelping litters	Control	38/39	35/37	10/10	10/10	11/11	11/11	19/20	17/18	151 litters/80 females
	Exptl	38/39	31/35	8/10	5/8	10/11	9/10	20/20	19/20	140 litters/80 females
Av. days mated before conception occurred[2]	Control	8.8	8.4	4.4	13.8	5.8	7.8	13.8	8.4	Av. 9.2 days (151 pregnancies)
	Exptl	9.8(37)	9.1(30)	15.8	10.6	16.7	12.3	13.3	14.1	Av. 11.7 days (138 pregnancies)
Pup Mortality										
Pups born[3] per litter	Control	10.0	7.9	9.5	9.7	8.5	11.8	10.0	9.9	1404 pups/150 litters av. 9.4
	Exptl	8.3	8.6	7.3	4.8	7.3	9.0	7.2	8.3	1121 pups/139 litters av. 8.1
Per cent found alive	Control	89.5	88.0	88.4	99.0	81.5	96.4	92.1	96.8	1287/1404 = 92%
	Exptl	87.0	93.1	98.3	62.5	94.5	100.0	78.4	89.2	997/1121 = 89%
Per cent of those found alive dying by 9th day	Control	29.9	17.0	28.4	4.1	31.9	9.8	20.6	32.0	323/1404 = 23%
	Exptl	27.8	13.7	20.7	33.3	20.5	7.4	48.6	29.8	276/1121 = 25%
Per cent pups discarded[4]	Control	1.3	2.2	0	7.2	0	4.5	2.6	3.2	38/1404 = 2.7%
	Exptl	0.3	2.3	0	0	1.4	2.5	1.4	1.2	12/1121 = 1.1%
Per cent survival[5] (21 days)	Control	58.2	69.6	60.0	94.4	59.6	85.0	71.4	59.7	917/1366 = 67%
	Exptl	57.4	77.3	77.6	29.2	73.6	92.4	28.9	58.2	694/1109 = 63%
Weanling mortality (21st-36th day)	Control	.....	0	0	0	0	0	0	0	0
	Exptl	.....	37	3	3	0	0	0	0	43[6]
Weaning Data										
No. litters weaned	Control	26/38	24/35	8/10	10/10	7/11	10/11	16/19	11/17	112/151 = 74%
	Exptl	25/38	27/31	7/8	2/5	7/10	8/9	7/20	13/19	96/140 = 69%
Av. pups in litters weaned	Control	8.4	7.8	7.1	8.5	8.0	9.1	8.1	8.4	917 pups/112 litters av. 8.2
	Exptl	7.2	7.5	6.4	3.5	7.6	9.1	5.9	7.1	694 pups/96 litters av. 7.2
Av. male weaning wts. (21 days) gm[7]	Control	45.8(112)	50.6(92)	47.7(26)	44.5(43)	45.8(35)	42.5(44)	42.2(54)	46.8(53)	46.1 (459)
	Exptl	42.5(85)	43.8(100)	46.3(26)	57.0(3)	44.2(28)	44.6(32)	49.9(28)	49.7(54)	45.5 (356)
Av. female weaning wts. (21 days) gm[7]	Control	43.8(106)	47.8(96)	45.5(31)	43.2(42)	42.1(21)	40.2(47)	40.6(76)	45.3(39)	43.8 (458)
	Exptl	42.9(96)	42.3(102)	46.6(19)	57.0(4)	42.1(25)	44.5(41)	44.9(13)	48.2(38)	44.6 (338)

[1] The data indicate proved fertility. The rotational mating procedure used did not afford equal or adequate opportunity to all males to demonstrate fertility. Hence, failure to produce a litter does not necessarily indicate a lack of fertility.

[2] The number of conceptions used to calculate the averages is identical with the number of conceptions listed under "females whelping litters" except in the experimental group, parent generation, both first and second litters, where one female was left out for each because of unusually long periods before conception occurred (69 days and 65 days, respectively).

[3] Pups "born" refers to the number of pups counted upon initial discovery of litters (checked daily).

[4] Litters were reduced to 10 pups between the 4th and 9th day.

[5] "Per cent survival" = [(total pups weaned) ÷ (total pups born − pups discarded)] × 100.

[6] All of these occurred on diets deficient in vitamin E. This number does not reflect an uncomplicated effect of the experimental diet.

[7] Figures in parentheses are the total number pups represented in the averages.

The first litters produced by both groups of parent generation animals were comparable. The only statistically significant difference observed was a smaller number of pups counted for females on the experimental diet. A rather high early mortality was observed in both the control and experimental litters, the cause of which is unknown.

In the second litters from parent generation animals fewer pups were born, but the early mortality declined. The per cent survival increased and was the highest for the experimental group.

The average weaning weights of all pups born to those parent generation females which weaned two[1] litters each were significantly higher for the control group than for the experimental group. The difference in weaning weights was small and not quite significant in the first litters; the difference in the second litters was larger and highly significant. Second litter weaning weights were larger than for first litters with the exception of the experimental female weanlings and the difference was significant.

The most obvious difference between the control and experimental pups from the parent generation appeared about the time of weaning, or shortly thereafter, when a considerable number of the second litter pups in the experimental group died. These deaths often occurred without observable symptoms and with little or no loss of body weight. In other animals, symptoms observed were marked edema of the face, ruffled hair coat, general incoordination, spastic hopping gait, and sometimes complete loss of movement with dragging of the hind quarters. Those pups most severely affected often became completely prostrated a short time before death. The most striking aspect was the marked suddenness of onset. Litters affected were usually of good weights and would appear perfectly healthy a few hours prior to the time when first symptoms and deaths were noted. In no case were these symptoms noted in the control group.

The above condition was entirely unexpected and most first litter pups were discarded at weaning (21 days) before it became evident. Two experimental first litter pups showed slight symptoms of a seemingly transient nature which were followed by apparently complete recovery. Sixteen pups of the second litters of the experimental parent generation were not kept beyond the 21st day. However, because of abnormalities in these pups, all the pups in subsequent litters were maintained for at least a week after weaning on their respective diets and then for another week on a stock diet before discarding.

Thirty-one of the 202 experimental pups (the first generation) were sacrificed for either histopathological or hematological examination shortly after weaning, and 2 litters totaling 16 pups were discarded when weaned on the 21st day. Some of these pups were in apparent good health and others were in a terminal condition. Forty-three pups died during the weaning period. Six died during the 18-21 day interval and 37 died within 10 days during the two week observation period after weaning. All deaths occurred in 14 of the 27 litters weaned. Pups in two other litters showed symptoms but did not die. Usually only a few pups in an affected litter would die and in no case did all die. It is possible that if the 31 pups sacrificed for histopathology and hematology and the 16 pups discarded at weaning had been given an opportunity to live for two additional weeks for observation, the total mortality of weanlings (21st to 36th day) might have been higher than the 37 deaths out of 202 pups weaned (or 18%) indicated in Table 5. The surviving animals discarded after the 36th day generally appeared normal in all respects.

The abnormality described above was strikingly similar to that first described by Evans and Burr *(6)* in the suckling young of female rats being maintained on a vitamin E low diet. Their observations have been confirmed repeatedly by others. The efficacy of synthetic DL alpha tocopherol in preventing the condition was demonstrated later *(1, 2, 8)* and has been confirmed in many laboratories. The probability that the second litter sucklings and weanlings were suffering from the characteristic muscular dystrophy syndrome (commonly referred to as nutritional muscular dystrophy) known to result from a marginal vitamin E intake was enhanced by the observation of a high degree of hemolysis of the red cells of the parent generation experimental animals by dialuric

---

[1] For convenience in statistical treatment only those rats were used which had produced two litters. The average weaning weights were similar to those in Table 5.

acid, using the technique reported by Rose and Gyorgy *(20)*. The control animals showed no appreciable hemolysis. Results of the dialuric acid hemolysis tests are reported in more detail under Hematology and Blood Analyses.

First generation. The above observations indicating a probable marginal vitamin E intake for the experimental animals led to modification of the procedure originally planned for the first generation animals in that the experimental and control animals were subdivided into unsupplemented and supplemented groups (one-half of each sex). Shortly after weaning, animals were selected from the second litters whelped by parent generation females. Where possible 2 males and 2 females were selected from each litter, one male and one female weanling going to each group. Thus the subgroups represent mostly a series of littermate pairs. Only healthy-appearing, representative pups were selected. Extremes in size were avoided. Supplementation was started just prior to mating at 14-15 weeks of age. The supplement consisted of 10 mg. synthetic DL alpha tocopherol dissolved in 0.1 ml. wheat germ oil administered orally to each animal about 8:00 A.M., Monday through Friday. Since the daily feeding usually was made about 3:00 P.M., possibility for the diet to destroy any of the supplemental DL alpha tocopherol by contact within the stomach or intestines was minimized.

The reproductive data for first generation animals tabulated in Table 5 indicate that a reduced degree of fertility probably existed in the unsupplemented experimental animals. There is also an indication that supplementation improved the fertility of the experimental animals, particularly during the period of second litter matings. The fertility of the control group remained high and unaffected by supplementation.

The survival to weaning of pups of the supplemented, experimental, first generation litters excelled that of the controls. Symptoms and mortality (three pups after weaning) were observed in 3 of the 8 first litters born to unsupplemented, experimental, first generation females.

The performance of the first generation in producing second litters was excellent for all animals except those of the unsupplemented experimental subgroup. Here, 3 females which weaned first litters failed to conceive a second time, 3 of 5 litters were lost, and only 7 of 24 pups born were weaned. All 7 pups became sick by the 23rd day, showing the characteristic symptoms previously described; 4 were sacrificed for histopathology and the remaining 3 died by the 26th day.

Second generation. The animals saved for the second generation were selected from the second litters whelped by supplemented first generation females of both dietary groups. Each second generation animal saved had been supplemented, starting about 18 days of age.

The reproductive success of the second generation (Table 5) indicates a continued high fertility for the control group and an equally high fertility for the experimental group. However, 13 out of 20 first litters born to the experimental group were lost prior to weaning age. The average number of pups born per litter was low. This poor performance did not occur in second litters. Second litters of control and experimental second generation females were comparable.

The results demonstrate the complete success of the supplement of DL alpha tocopherol in wheat germ oil in preventing the paralytic symptoms and sudden death observed in experimental weanling pups, thus affording direct evidence that the condition was nutritional muscular dystrophy.

Hematology and Blood Analyses

Routine hematological examinations (red cell count, white cell count, differential count and hemoglobin content) were conducted on 64 parent generation females (16 males and 16 females from each group) during the 11th experimental month. Fifty-six survivors of this sample were examined again during the 15th experimental month. Finally 39 survivors were examined after 20 months.

The blood picture did not present anything unusual for either dietary group. Such differences as did exist appeared incidental and small enough that statistical analysis was judged by the authors and statistician as unnecessary to establish a lack of any effect of irradiation of the meat component of the diet on blood morphology.

In addition to these planned hematological examinations, dialuric acid hemolysis

tests[a] were made where the vitamin E status of the animals was questioned. A total of 64 blood samples from 42 parent generation animals were subjected to this test from the 38th through the 55th week of experiment. All of the 31 samples from 20 control animals showed less than 7% hemolysis; 25 samples showed no hemolysis and the overall average hemolysis was 1%, thus showing no indication of deficiency. On the other hand, 32 of 33 samples from 22 experimental animals showed more than 81% hemolysis, averaging 91%, definitely indicating the probable existence of a tocopherol deficient state in these unsupplemented parent generation animals.

The test was applied also to 46 first generation animals (49 blood samples taken during the 20th-28th week on experiment). Twenty-four samples from 24 control animals showed no hemolysis, whether or not supplemented with DL alpha tocopherol in wheat germ oil. Likewise, 12 experimental animals receiving the supplement showed no hemolysis (12 blood samples) indicating that the supplementation corrects the deficiency. Thirteen samples from 10 unsupplemented experimental animals showed more than 67% hemolysis, averaging 93%.

When the test was applied during the 27th through the 33rd week on experiment to the second generation animals, all of which had been supplemented beginning about 18 days of age, single samples from 12 control and 14 experimental animals showed little or no hemolysis, thus again indicating the effectiveness of the supplementation in correcting the deficient state otherwise existing. The control bloods showed less than 7% hemolysis, averaging 0.8%; the experimental samples less than 15%, averaging 1%.

Mortality and Life Span

Data in Table 6 show that the survival of the parent generation animals was good for both the control and experimental dietary groups throughout the first year. Most of the mortality occurred during the last 6 months of the 2-year period. Approximately equal numbers of male animals survived the 2-year period in each group (5/30[b] controls and 7/30 experimentals). Greater numbers of female animals survived than males, and substantially more control females (17/30) than experimental females (9/30). The average life span was very good for both groups, although significantly shorter for both sexes of the experimental group.

First and second generation animals were sacrificed for gross pathology when reproduction was completed (after 30 weeks for males and 35 weeks for females). No first generation control animals died. Four experimental animals in the first generation died during the first week, possibly of vitamin E deficiency. The 4 dead were replaced with 4 additional weanlings from the same group, after which there was no further mortality.

In the second generation two control females died, in the 25th and 29th weeks respectively.

In the parent generation those animals which were observed to be in a "terminal condition," i.e., almost certain to die soon, were sacrificed, in many instances for reasons explained later. Although it was realized that the life span of animals so sacrificed may have been shortened artificially thereby, these animals were included with those found dead in the mortality data in Table 6.

Neoplasms

As expected in long term experiments, a few animals showed "enlargements" which might have been neoplasms or tumors, cysts, abscesses, etc. Table 7 shows that the neoplasms (confirmed by gross and/or histopathology) were distributed approxi-

---

[a] This test, described by Rose and Gygory *(20)*, correlates the degree of *in vitro* hemolysis by dialuric acid with the tocopherol status of the rat, deficient animals showing a high degree of hemolysis by dialuric acid. The authors are indebted to Dr. Catherine S. Rose for a personal communication detailing the technique and for providing the first sample of dialuric acid used in our tests.

[b] Ten of the original 40 animals started were not included, inasmuch as these animals were sacrificed routinely for histopathology and therefore did not have a chance to survive the entire two-year period.

TABLE 6

Summary of Mortality and Sacrifice

	Parent generation				First generation				Second generation			
	Males		Females		Males		Females		Males		Females	
	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl
No. of rats started	40	40	40	40	19	22	21	22	10	10	20	20
Cumulative mortality:[1]												
(A) end of 30 weeks	0	1	0	0	0	3	0	1	0	0	2	0
(B) end of 52 weeks	0	4	1	1	Sacrificed after 30 to 35 weeks							
(C) end of 78 weeks	8	11	1	8	....	....	....	....	....	....	....	....
(D) end of expt—104 weeks	25	23	13	21	....	....	....	....	....	....	....	....
Sacrificed for histopathology:												
(A) during expt	10	10	10	10	0	0	0	0	0	0	0	0
(B) end of expt	5	7	11	9	0	0	1	0	0	0	1	3
Sacrificed for gross pathology only at end of expt	0	0	6	0	19	19	20	21	10	10	17	17
Average days survival[2] (on expt) and standard error	614 ± 18.8	581 ± 24.9	685 ± 14.7	612 ± 21.8	....	....	....	....	....	....	....	....

[1] Includes those animals sacrificed in a terminal condition to obtain tissues for histopathology; does not include animals sacrificed routinely during and at end of experiment for histopathology.

[2] Exludes animals sacrificed routinely during experiment for histopathology, but includes those sacrificed in a terminal condition. Also excludes one animal in the experimental male group which died early (19 days), and another in the experimental female group which was killed accidentally (511 days). Potential life span was limited experimentally to 728 days. Life span of experimental animals was significantly shorter for both sexes ($P<0.05$).

mately equally between the control and experimental animals. The pathologist listed 6 animals with 6 malignant neoplasms and 9 animals with 12 benign neoplasms in the control group. In the experimental group there were 4 animals with 4 malignant neoplasms (one of which was questionable), 9 animals with 10 benign neoplasms (two of which were questionable), and one animal whose neoplasm could not be classified histologically because of accidental loss of the section. Most of the neoplasms occurred in 141 animals of the parent generation after 300 days on the experiment. The similar incidence and miscellaneous pattern in location and type point toward "spontaneous" origin completely unrelated to irradiation of the meat ingredient of the diet.

Special attention was given throughout the experiment to searching for possible neoplasms in both living and dead animals in order to minimize the possibility of overlooking any which occurred.

Gross and Histopathology

Equal numbers of males and females from each dietary group of the parent generation were selected by drawing numbers for gross and histopathological examination after 3 and 6 months on experiment (8 animals each time) and after 12 and 18 months (12 animals each time). On termination of the experiment, after 24 experimental months, all surviving animals in the experimental group (7 males, 9 females) and a comparable number of control animals were taken for histopathological examination. The remaining survivors (6 control females) were sacrificed and examined grossly.

In addition to planned sacrifices for histopathology, any animals found in a "terminal condition," i.e., almost certain to die soon, were, in many instances, sacrificed in order to obtain fresh tissue samples. The only exception regularly made was the exclusion of animals whose terminal condition appeared to be due obviously to severe chronic respiratory disease. In some instances, tissues appearing to be abnormal grossly were saved from animals found dead, and an attempt was made to obtain the best histological preparation possible from such material; particular attention in this regard was given to tissues which appeared possibly neoplastic.

When the unexpected symptoms and mortality were observed in the second litter offspring of the parent generation of experimental animals, special arrangements were made for the histopathological examination of 6 control and 18 experimental weanlings. Most of the animals examined were sacrificed, although some had died just before examination. In addition, a histopathological examination was made of a sample more uniform in number and distribution and representing the second litters of the first and second generations. Six supplemented and 6 unsupplemented weanlings in each dietary group of the first generation and 14 control and 11 experimental weanlings of the second generation (all supplemented) were examined.

After the conclusion of the reproductive phase (35 experimental weeks for the females and 30 for the males) the first and second generation adults were sacrificed and examined grossly. In addition the liver, spleen, kidneys, and testes of most animals were weighed.

The following tissues were saved routinely from most animals selected randomly for histopathological examination: reproductive organs, kidneys, adrenals, spleen, pancreas, stomach, small intestine (duodenum, ileum and/or jejunum), colon, liver, lungs, mediastinal structures, lymph nodes (usually mesenteric and/or cervical), thymus, and bone marrow. Skeletal muscle was usually included subsequent to the observation of paralytic symptoms in weanlings. Frequently but less regularly examined were the cecum, urinary bladder, salivary glands and central nervous system. Occasionally other tissues were sampled, e.g., all neoplasms were included, as well as still other apparent gross abnormalities at the discretion of the pathologist conducting the autopsy. In animals found dead histopathology, if done, was limited usually to those tissues appearing to be abnormal grossly. Differential marrow smear counts were made on a substantial number of animals.

Nearly all histopathological and most of the gross examinations were made by Dr.

TABLE 7
Summary of neoplasms

Group	Histological classification and description		Location	Days on experiment	
				First noted[1]	Autopsied
Parent generation					
Control males	Malignant	Pharyngeal carcinosarcoma	Pharynx	342	368
	Malignant	Small mixed tumor (carcinomatous)	Parotid	441	476
	Benign	Large cortical adenoma	Adrenal	—	554
	Malignant	Mediastinal lymphosarcoma (4 g.)	Anterior mediastinum	—	634
Control females	Malignant	Mixed tumor in orbits, bilateral, infiltrating brain	Orbital	279	348
	Malignant	Large retroperitoneal fibrosarcoma	Peritoneal cavity	—	592
	{ Benign	Two fibro-adenomas (33 & 115 g.)	{ Mammary (two)	524	616
	{ Benign[6]	Small dermoid cyst	{ Thorax		
	Malignant	Fibrosarcoma	Peritoneal cavity	679	711
	Benign	Fibro-adenoma (6 g.)	Right axilla	707	729[2]
	Benign	Fibro-adenoma (125 g.)	Mammary	581	729[2]
	Benign	Fibro-adenoma (12 g.)	Mammary	686	733[2]
	Benign	Adenoma (17 g.)	Mammary	672	734[2]
	Benign	Two adenomas (5 & 6 g.)	Mammary (two)	469	735[2]
	Benign	Fibro-adenoma (11 g.)	Mammary	672	735[2]
Experimental males	Malignant	Small neurofibrosarcoma	Right face	336	449
	Benign?	Fibroma (possibly fibrosarcoma) ((11 g.)	Thorax	—	634
	Benign	Fibroma (medium size)	Side of head	236	707
Experimental females	Benign	Fibro-adenoma (112 g.)	Mammary	315	456
	{ Benign	Fibro-adenoma (166 g.)	{ Mammary	315	456
	{ Benign?[4]	Small cellular fibroma	{ [3]		
	Benign	Fibro-adenoma (294 g.)	Mammary	399	546
	Benign	Adenoma (95 g.)	Mammary	497	562
	Benign	Fibro-adenoma (2 g.)	Ovary[3]	431	575
	Malignant	Large carcinoma, undifferentiated	Peritoneal cavity	—	594
	Malignant	Fibrosarcoma (175 g.)	Perineal region	644	705
	Benign	Fibro-adenoma (18 g.)	Mammary	693	734
First generation		No neoplasms noted in any groups			
Second generation					
Control females	Benign	Fibro-adenoma (40 g.)	Mammary	188	252
Experimental females	Benign	Small fibro-adenoma	Mammary	—	245
	Malignant?	Lympho-epithelionic tumor (8 g.)	Anterior neck region	140	245
	[5]	Small cystic tumor	Left masseter muscle	—	245

[1] Dash indicates neoplasm was not noted prior to autopsy.
[2] Although experiment was "terminated" at 728 days, some animals were continued on test for a short time until it was possible to sacrifice them.
[3] No autopsy by pathologist; location was not recorded.
[4] Classification questionable without knowledge of location.
[5] Section of tumor tissue was lost accidentally, hence no histopathological examination to permit classification.
[6] Brackets denote more than one type of neoplasm in the same animal.

John B. Fuller.[1] He usually sacrificed the animals (taken to his laboratory for this purpose), and reported his observations under autopsy code numbers which had been assigned to prevent any knowledge on his part of the identity and treatment of each specific animal examined; in addition, he had only a very general knowledge of the nature of the experiment prior to completing his observations.

The examination of hemotoxylin and eosin preparations of central nervous system tissues by Dr. Fuller was supplemented by further examination by Dr. Arthur Arnold,[2] using techniques specially applicable to such tissues. This was done as an exploration in recognition of the fact that the usual hemotoxylin and eosin tissue preparations may not reveal all pathology in such tissues. A small cross-section of 20 animals covering most groups was so examined with negative findings.

A total of 432 animals were autopsied during the course of the experiment. A gross examination only, including weights of principal organs in many instances, was made on 249 of the 432 animals. Such examinations were limited mostly to weanlings and young adults and were negative in that they revealed no unusual gross lesions which might have been evidence of harm to the animal resulting from the consumption of irradiated meat. For the sake of brevity these negative data have not been included in this publication.

A total of 2571 tissues were examined histologically from the remaining 183 animals brought to autopsy. This number includes those which were selected randomly for such examinations, and other animals in which a gross examination had revealed unusual lesions requiring histopathological characterization.

As shown in Table 8, most of the tissues examined were normal (2037/2571 total; 970/1224 control and 1067/1347 experimental). It so happened that the percentage of normal tissues was the same (79.2%) for both the control and experimental groups.

The 534 tissues which were not normal showed a total of 632 histological deviations.[3] Although it is not practical to tabulate here all of the deviations observed, Table 8 does show those which occurred three or more times in any one or more of the groups and subgroups listed across the top of the table. Thus 412 deviations are classified out of a total of 632 which were observed in the 2571 tissues examined from 183 animals. The degrees of severity for the deviations varied from slight to quite severe and are not shown.

The 412 deviations tabulated as occurring most frequently are classified into 18 types according to the nature and location of the deviation. On a similar basis, the 220 deviations lumped into the category *other miscellaneous* represent an additional 107 types.

The deviation occurring most frequently was hemosiderosis of the spleen; it was found in nearly all adult animals, both control and experimental; its significance is obscure.[4] Testicular atrophy, decreased spermatogenesis, extramedullary hematopoiesis in the spleen, and fatty livers were the deviations next most frequent in occurrence; again the distribution between the control and experimental groups was nearly the same. These 4 deviations alone account for 230 of the 632 deviations observed (36.4%). Other deviations occurring in adult animals were spontaneous, heterogeneous changes

---

[1] Dr. Fuller, Associate Professor of Pathology and Assistant Director of the Hospital Laboratory, Research & Educational Hospital, College of Medicine, University of Illinois, 808 Wood Street, Chicago, Illinois, conducted and reported the pathological examinations of the animals and tissues, including differential marrow smear counts on some animals.

[2] Dr. Arthur Arnold, Assistant Professor of Neurology and Neurosurgery, Department of Neurology and Neurological Surgery, Neuropsychiatric Institute, College of Medicine, University of Illinois, 912 Wood Street, Chicago, Illinois, conducted and reported special histopathological examinations of central nervous system tissues.

[3] Deviation as used here does not necessarily imply pathological significance, but represents any condition which the pathologist saw fit to describe rather than calling it "normal." A single tissue may show more than one deviation in some instances.

[4] Moderate to severe hemosiderosis of the spleen was found in 4 rats which had been maintained on a stock diet for 35-40 weeks. The animals were obtained from the same source nearly two years later and raised in the same animal room. The implication is that hemosiderosis of the spleen of adult rats may be characteristic of this strain and/or this environment.

TABLE 8

Summary of histopathological observations[1]

	Parent generation												First Generation		Second generation						Third Generation		Totals		
	3 months		6 months		12 months		18 months		24 months		Terminal or died[2]		Weanlings		Weanlings				Adults[3]		Weanlings				
															Unsupplemented		Supplemented		Supplemented						
	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl	All Groups
Number animals examined	4	4	4	4	6	6	6	6	17	16	16	17	6	18	6	6	6	6	1	3	14	11	86	97	183
Number tissues examined	65	62	69	63	98	98	106	83	238	222	99	113	120	329	97	80	106	89	18	47	208	161	1224	1347	2571
Number tissues normal	60	57	62	52	83	79	73	59	152	137	24	54	119	300	93	56	101	82	10	40	193	151	970	1067	2037
Number deviations observed	5	5	7	12	16	22	43	30	113	102	90	72	1	28	4	25	5	7	9	10	16	10	309	323	632
Hemosiderosis—spleen	4	4	4	4	6	6	6	6	15	16	6	6	0	2	0	0	0	0	1	3	1	0	43	47	90
Hemosiderosis—lymph nodes—various sites	0	0	0	3	1	2	1	2	2	4	0	1	0	0	0	0	0	0	0	0	0	0	4	12	16
Testicular atrophy and decreased spermatogenesis	0	0	0	0	1	3	6	4	9	12	9	5	0	3	0	0	0	0	0	0	0	0	25	27	52
Extramedullary hematopoiesis—spleen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	6	5	5	1	3	13	10	23	24	47
Fatty change—liver	1	1	0	0	0	0	3	1	12	8	4	5	1	2	0	1	0	2	0	0	0	0	21	20	41
Bronchitis, pneumonitis or pneumonia	0	0	0	0	0	0	4	3	9	12	5	3	0	1	0	0	0	0	1	1	0	0	19	20	39
Degenerative changes—skeletal muscle	—	—	—	0	0	0	1	2	1	4	0	0	0	12	0	6	0	0	0	0	0	0	2	24	26
Neoplasms—benign	0	0	0	0	0	0	0	0	7	1	4	8[5]	0	0	0	0	0	0	1	1[4]	0	0	12	10	22
Neoplasms—malignant	0	0	0	0	0	0	0	0	1	0	5	3	0	0	0	0	0	0	0	1[6]	0	0	6	4	10
Atrophy—spleen	0	0	0	0	0	0	0	0	7	5	1	0	0	0	0	0	0	0	0	0	0	0	8	5	13
Atrophy—liver	0	0	0	0	0	0	0	0	2	1	4	3	0	0	0	2	0	0	0	0	0	0	6	6	12
Atrophy—skeletal muscle	—	—	—	0	0	0	0	0	2	2	3	1	0	0	0	0	0	0	0	0	0	0	5	3	8
Atrophy—ovary	0	0	0	0	0	0	0	0	5	2	1	0	0	0	0	0	0	0	0	0	0	0	6	2	8
Pyelonephritis	0	0	0	0	0	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	6	0	6
Ovarian cysts	0	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	1	0	0	0	5	1	6
Reticular hyperplasia—spleen	0	0	0	0	0	0	0	1	1	4	0	0	0	0	0	0	0	0	0	0	0	0	1	5	6
Enlarged spleen	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	3	2	5
Thickening of the media of central arteries of spleen	0	0	0	0	0	0	3	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	5	0	5
Other miscellaneous deviations	0	0	3	5	8	11	17	10	35	31	40	35	0	8	0	10	0	0	4	1	2	0	109	111	220

[1] A "zero" signifies tissues were examined but deviation was not found. Dashes indicate that the tissues were not examined.
[2] Histopathological examination of tissues of animals found dead, when done, was ordinarily limited to those tissues appearing abnormal grossly.
[3] These animals were examined histologically only because neoplasms were observed grossly.
[4] One additional neoplasm was observed grossly, but could not be classified histologically because the section was lost accidentally.
[5] Benign nature of two neoplasms was questionable.
[6] This neoplasm was a questionable low grade malignancy.

having no pattern or trends in either the control or experimental dietary groups. The number and variety of such changes increased markedly with advancing age.

There were few unexplained abnormalities in weanling animals, and again an absence of any pattern or trends with one outstanding exception. The first generation experimental weanlings, in which a high incidence of muscular weakness and incoordination with sudden death had been observed grossly, showed a correspondingly high incidence of degenerative changes histologically in the skeletal muscles (12 out of 18 animals examined). Unsupplemented second generation experimental weanlings likewise showed mortality and gross muscular weakness as well as histological degenerative changes in the skeletal muscle; these abnormalities were prevented in the subgroup of second generation experimental weanlings receiving the supplement of DL alpha tocopherol in wheat germ oil. Likewise, these abnormalities were not observed in any of the third generation experimental weanlings, all of which received the supplement.

There were no real differences in organ weights between the control and experimental groups except for testes weights of the first generation experimental adults without supplementation. Supplementation increased the testes weights of the first generation experimental animals substantially, although they were still somewhat smaller than those of the controls. The testes weights of the second generation experimental animals, all of which received the supplement, were equal to the testes weights of the corresponding control animals.

TABLE 9

Testes weights at 35 weeks on test

	Number of animals		Average body weight		Average testes weight		Average % of body weight	
	Control	Exptl	Control	Exptl	Control	Exptl	Control	Exptl
			grams		*grams*			
First generation								
Unsupplemented	7	7	629	544	3.27	1.52	0.53	0.28
Supplemented	6	7	597	516	3.25	2.12	0.55	0.41
Second generation[1]	7	10	539	526	3.41	3.44	0.64	0.66

[1] All second generation animals were supplemented with DL alpha tocopherol.

Differential marrow smear counts were completed on 72 animals, 34 control and 38 experimental. The distribution by age and group in the parent generation was as follows: 6 months—control 4, experimental 4; 12 months—6, 6; 18 months—6, 5; 24 months—14, 14. Likewise, the distribution in the first generation was: weanlings—4, 9. The only consistent patterns noted in the differential marrow smear counts were (a) a progressive increase in myeloid/erythroid ratios owing to a progressive decrease in the proportion of red cell precursors associated with aging of the rats, and (b) a marked increase in the proportion of polymorphonuclear leukocytes, metamyelocytes and myelocytes in all rats with acute and subacute infections, such as pneumonitis, peritonitis associated with abdominal or pelvic tumors, etc. There were no indications that the deviations observed were related to the irradiation of the meat ingredient of the experimental diet. Tabulation of these negative data has been omitted for brevity.

DISCUSSION

It is clearly evident that both the control and experimental diets fed in this experiment supported an unusual rate of growth, leading also to an unusually large adult size (marked obesity) of the control males, particularly later in the life span. The females did not tend toward obesity as early as the males, usually becoming obese during the second year. It is believed that the tendency toward obeseness was a result of the highly

palatable, high meat, high fat diet fed *ad libitum* in conjunction with the limitation of activity associated with confinement in a cage.

The slightly smaller adult size of the experimental animals, males in particular, is probably correlated with the slightly poorer utilization of food observed for the experimental animals. The reason for the poorer utilization of food by the experimental animals is not determinable from the data. Although the irradiated meat was the only significant source of protein in the experimental diet, it is believed that a diminished nutritional quality of the meat protein by irradiation is not a probable explanation, since a preliminary experiment with beef hamburger irradiated with 5 million rep showed only a slight, probably insignificant, effect on protein quality evaluated under the stress conditions of a 5% protein level (based on diet solids). The irradiation process may have slightly lowered the overall nutritive value of the beef hamburger including caloric utilization. Since the hamburger is a major ingredient in the diet, any general effect on nutritive value would be reflected in a slightly lowered efficiency of utilization of the diet; conventional heat sterilization of the beef hamburger instead of irradiation might have produced a similar, perhaps larger, effect. The unusual rate of growth of the experimental animals, as well as the trend toward better growth and food utilization in subsequent generations rather than a cumulative decline, would seem to remove any real question regarding the wholesomeness of the irradiated beef.

The comparison of the reproductive performance of the experimental animals with that of the control is complicated to some degree by the demonstration that the tocopherol content of the experimental diet was reduced to a submarginal level by the irradiated meat component. However, the muscular dystrophy syndrome of the experimental sucklings and weanlings, which was the outstanding difference in reproductive performances, was shown clearly to be a result of the submarginal DL alpha tocopherol intake. The complete prevention of the condition by an oral supplement of DL alpha tocopherol shows that it probably was caused by a deficiency and not by a toxic substance.

The tendency toward declining fertility of the experimental group in the first generation was, as might be expected, reversed to a considerable extent in regard to litters born by supplementation with DL alpha tocopherol. The number of pups born per litter, another criterion of fertility, was improved also by the supplementation. The success in weaning pups was better. While the data demonstrate the role of DL alpha tocopherol as regards these criteria, there is also the possibility that other factors such as a slight decrease in general nutritional availability or some other effect of irradiation of the meat might have been involved also. In this connection, the overall experience of reproductive success for the control and experimental groups has been compared by groups in the last column of Table 5 without regard to generation, litter, or supplementation. Such a comparison for the considerable numbers of animals involved leaves little doubt of a substantially better reproductive performance of the control group in production of weanlings. However, the overall reproductive success of the experimental group is reasonably good in spite of the demonstrated effects of DL alpha tocopherol deficiency. The failure to equal

completely the control group in reproductive success may be due to the same factor(s) responsible for the slightly decreased efficiency of food utilization and smaller size of the experimental groups, thought to be, as previously discussed, possibly due to a slightly lower nutritional value. Retorting the meat instead of irradiating it might have caused a similar effect.

The survival of both control and experimental animals was very good, although the average life span of the experimental group was 5 to 7% shorter than the life span of the control for both sexes in the parent generation. No explanation is suggested by the results to account for this difference; it is not known whether the lower tocopherol content of the experimental diet could have caused a shortening of the life span. In this connection it should be emphasized that none of the parent generation animals were supplemented with DL alpha tocopherol at any time.

Nearly all of the histological abnormalities observed in both groups were those heterogeneous, spontaneous changes which would be expected in any aging group of rats. The only specific histological differences showing up in the experimental group when compared with the control were those of the condition which Mason *(15)* describes as early or explosive type of nutritional muscular dystrophy. This condition is known to result from a partial deficiency of DL alpha tocopherol and this, rather than the irradiated meat *per se*, was demonstrated to be the cause of the nutritional muscular dystrophy observed in this experiment. The incidence of benign and malignant tumors was quite similar for both groups, thus indicating that irradiation of the meat did not produce carcinogens demonstrable under the conditions of this experiment.

The most logical cause for the partial DL alpha tocopherol deficiency induced by the diet containing irradiated beef is destruction by oxidation of part of the tocopherol included in the diet. The existence of oxidizing conditions under the influence of irradiation has been demonstrated repeatedly and, more specifically, increased peroxide values of fats or fatty components of foods and linoleic acid autoxidation with increased peroxide formation have been reported several times *(3, 4, 9, 16, 17, 19)*. Exploratory peroxide analyses of samples of irradiated meat used in this experiment, as well as other samples of irradiated meat not used in this experiment, have indicated slight but definite elevations of the peroxide values. Exploratory ultra-violet spectra of the fat rendered from irradiated meat have indicated shifts in the spectra toward increased general absorption characteristic of oxidized fats. It is probable that further increases in peroxide value may have occurred during the prolonged storage periods (as long as 6 months at −18°) necessary to insure the continuity of supply for the experiment. The mixing procedure wherein the diets were mixed and stored for periods up to 12 days provided ample opportunity for destruction of the added tocopherol by contact with the irradiated meat component. It was also found that a substantial loss of tocopherol occurred during the mixing into the diet premix of the wheat germ oil used as the source of tocopherol. The approximate extent of the destruction was indicated by analyses of the wheat germ oil and the finished diets for total tocopherol content by the method of Emmerie and Engel *(5)*. The total tocopherol content of

the wheat germ oil was found to be 2.5 mg. per g.; thus the tocopherol added to each 100 g. of diet solids was about 2.6 mg. The total tocopherol contents for the control and experimental diets 3 days after preparation were found to be approximately 1.2 mg. and 0.3 mg. respectively per 100 g. of diet solids representing retentions of approximately 46% and 12% respectively, of the amount added initially. About 21% of the tocopherol added disappeared during preparation of the premix. During mixing the premix with raw beef hamburger to prepare the control diet an additional 33% of the tocopherol disappeared, compared with an additional 67% disappearing during mixing of the premix with the irradiated beef hamburger to prepare the experimental diet. Destruction of the tocopherol by the irradiated meat component of the experimental diet was just sufficient, apparently, to make the total DL alpha tocopherol content of the experimental diet submarginal for rats. The complete prevention of the nutritional muscular dystrophy and improvement of fertility of the experimental group following oral supplementation with DL alpha tocopherol demonstrate that irradiation was involved indirectly and only to the extent of producing conditions which enhanced tocopherol losses in the diet use.

SUMMARY AND CONCLUSIONS

Raw ground beef has been irradiated with two million rep of electrons (cathode rays or beta rays) and compared with similar raw ground beef not irradiated by feeding to albino rats through three generations (a total of 2685 animals). About two-thirds of the diet as fed was composed of meat, equivalent to about 45% of the diet solids or 60% of the calories. Results regarding growth, adult size, efficiency of food utilization, reproduction, hematology, survival, pathology, and neoplasms have been described.

The animals fed the irradiated beef over a life span (two years) and through successive generations performed very well. Once initial marginal deficiencies of vitamin E (due to destruction during mixing and storage of the diet) were corrected, no major differences existed between the control and experimental groups. No gross or histopathological lesions attributable to irradiation of the beef were observed. The small and occasionally statistically significant decreases in growth, food efficiency, reproduction, adult body size and survival were considered to be due to slightly decreased nutritional quality, similar to that which occurs during heat sterilization, and not an indication of toxic effects.

It is concluded that the data presented herein provide substantial evidence that treatment of beef muscle with a sterilizing dose (two million rep) of radiation of high velocity electrons does not significantly impair nutritional value or wholesomeness.

Acknowledgments

The authors wish to express their thanks to the following persons for their assistance with certain parts of the experimental work reported: C. M. Zoubek, T. R. Simonson and H. J. Czarnecki for preparation, irradiation, shipment and storage of the considerable quantities of control and irradiated meat required; Dr. H. P. Andrews for the statistical analyses; J. F. Beuk, Dr. John Hogan and Dr. F. W. Chornock for carrying

out the dialuric acid hemolysis tests and tocopherol analyses; M. G. Anderson, G. A. Werner and Jacquelin Lambert for hematology; B. N. Rockwood for proximate analyses of diets and ingredients; K. H. Spitzmueller for preparation of some of the dialuric acid used for hemolysis tests; Dr. A. L. Lingard for ultra violet spectral analyses of fats from irradiated meat.

LITERATURE CITED

1. Barrie, M. M. O. Vitamin E deficiency in the suckling rat. *Nature*, **142**, 799 (1938).
2. Demole, V., and Pfatz, H. Vitamin E deficiency and neuromuscular impairment in rats, and treatment with synthetic vitamin E. *Schweiz. Med. Woch.*, **69**, 123-125 (1939).
3. Dubouloz, P., Dumas, J., and Vigne, J. Presence of lipide peroxides in the skin after action of various physical agents. *Compt. rend. soc. biol.*, **144**, 1080-1081 (1950).
4. Dunn, C. G., Campbell, W. L., Fram, Harvey, and Hutchins, Ardelia. Biological and photo-chemical effects of high energy, electrostatically produced roentgen rays and cathode rays. *J. Applied Phys.*, **19**, 605-616 (1948).
5. Emmerie, A., and Engel, Chr. Colorimetric determination of tcoopherol (vitamin E). *Rec. trav. chim.*, **57**, 1351-1355 (1938).
6. Evans, H. M., and Burr, G. O. Development of paralysis in the suckling young of mothers deprived of vitamin E. *J. Biol. Chem.*, **76**, 273-297 (1928).
7. Foster, F. L., Dewey II, D. R., and Gale, A. J. Van de Graaff accelerators for sterilization use. *Nucleonics*, **10**, No. 10, 14-17 (1953).
8. Goettsch, Marrianne, and Ritzmann, Johana. The preventive effect of wheat germ oils and of α tocopherol in nutritional muscular dystrophy of young rats. *J. Nutr.*, **17**, 371-381 (1939).
9. Hannan, R. S., and Boag, J. W. Effects of electronic irradiation of fats. *Nature*, **169**, 152-153 (1952).
10. Huber, Wolfgang. Electronic preservation of food. *Electronics*, **21**, No. 3, 74-79 (1948).
11. Jones, J. H., and Foster, Claire. A salt mixture for use with basal diets either low or high in phosphorus. *J. Nutr.*, **24**, 245-256 (1942).
12. Knowlton, J. A., Mahn, G. R., and Ranftl, J. W. Radiation sterilization III, The resonant transformer: a source of high-energy electrons. *Nucleonics*, **11**, No. 11, 64-66 (1953).
13. Lawton, E. J., and Balwit, J. S. Ionization Chambers for Measuring Cathode-Ray Dose Rate. (Unpublished Report N. RL-618, General Electric Research Laboratory, Nov., 1951).
14. Lea, D. E. *Actions of Radiations on Living Cells.* 1947, Macmillan and Co., New York, pages 8, 16.
15. Mason, K. F. Experimental muscular dystrophy. *Proceedings of the First and Second Medical Conference of the Muscular Dystrophy Association of America, Inc.*, pages 25-31 (1951-52).
16. Mead, J. F. The irradiation-induced autoxidation of linoleic acid. *Science*, **115**, 470-472 (1952).
17. Polister, B. H., and Mead, J. F. Effect of certain vitamins and antioxidants on irradiation-induced autoxidation of methyl linoleate. *J. Agr. Food Chem.*, **2**, 199-202 (1954).
18. Proctor, B. E., and Goldblith, S. A. Food processing with ionizing radiations. *Food Technol.*, **5**, 376-380 (1951).
19. Proctor, B. E., and Goldblith, S. A. Electromagnetic radiation fundamentals and their applications in food technology. *Advances in Food Research*, **3**, 119-196 (1951).
20. Rose, C. S., and Gyorgy, P. Hemolysis with alloxan and alloxan-like compounds, and the protective action of tocopherol. *Blood*, **5**, 1062-1074 (1950).
21. Siri, W. E. *Isotopic Tracers and Nuclear Radiations with Applications to Biology and Medicine.* 1949, McGraw-Hill Book Co., pp. 410, 413.

TOXICOLOGICAL DETERMINATION OF SUITABILITY OF FOOD ADDITIVES*

HARRY J. DEUEL, JR.
Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles, California

(Manuscript received June 29, 1954)

The question as to what chemicals may be permitted as food additives has assumed increasing importance. Many products which have been developed have been useful in extending the shelf life of the product or in enhancing the palatability, convenience, and attractiveness of prepared foods. Substances which act as food preservatives may well be expected to result in increased economy, not only for the food producer, but also for the consumer.

The new chemicals employed in increasing the usefulness of foods are not confined to those used in prepared foods. In fact, the problem of chemicals in foods is related, to an even greater extent, to the use of fungicides, herbicides, insecticides, and rodenticides, usually grouped under the term "pesticides." Thus, the widespread use of DDT, chlordane, selenium, phenylmercury compounds, and benzene hexachloride has posed problems in the food industries. It is generally agreed that many crops cannot be efficiently produced without the use of insecticides; in fact, our ever-increasing production of foodstuffs is closely related to the better protection from insect pests afforded by the newer pesticides. Likewise, because of the acquired tolerance which is developed toward these compounds, new ones must continually be supplied to replace those no longer effective. It would therefore appear that an understanding of the toxicological action of the various insecticides must necessarily be a present and continuing research problem.

Another cause for concern involves the use of hormones in the field of food production. Chemicals of this nature have been used for the purpose of improving the quality, and in some cases the quantity, of the foods produced. It is still uncertain whether or not the consumption of such food may involve a health hazard. As an example, diethylstilbestrol has been employed for fattening and tenderizing poultry and livestock. It is estimated that, in 1950, approximately 30,000,000 chickens were treated with 15 mg. diethylstilbestrol pellets. The use of the preparation is reported to be increasing.

The 81st Congress of the United States appointed a Committee to investigate *The Use of Chemicals in Foods;* this was commonly known as the Delaney Committee, after its chairman, James J. Delaney of New York. Other members of the committee included E. H. Hedrick (West Virginia), Paul C. Jones (Missouri), A. L. Miller (Nebraska), and Gordon L. McDonough (California). The scope of the investigation by this committee was

---

* Presented at the Fourteenth Annual Meeting of the Institute of Food Technologists, Los Angeles, Calif., 1954. Contribution No. 387 of the Department of Biochemistry and Nutrition, University of Southern California.

extended by the 82nd Congress to include cosmetics. Public hearings were held on 59 days during the period 1950-52, and 217 witnesses discussed the use of chemicals in fertilizers, in food production and processing, as well as their distribution in foods and cosmetics. The report of the Delaney Committee comprises 1931 pages but it has been published in a short condensed version *(2)*.

An unofficial group concerned with the safety of chemicals in foods is the Food Protection Committee of the Food and Nutrition Board of the National Research Council *(7)*. This committee attempted to formulate guiding principles and to specify adequate controls to assure the safety of chemicals employed in food technology. It also assembled and evaluated scientific information. This has been used to advise interested parties concerning questions on the safety of specific chemicals. Smyth has recently discussed the toxicological aspects of food additives *(14)*.

OBTAINING EVIDENCE OF HARMLESSNESS—SOME SELECTED EXAMPLES

The fact that the problem of chemicals in foods is a serious one at the present time is indicated by the large number of specific cases which have come to the attention of the U. S. Food and Drug Administration in recent years. As a result of these investigations, such chemicals as nitrogen trichloride (agene), thiourea, *p*-phenylethylurea, lithium chloride, mineral oil, monochloracetic acid, dehydroacetic acid and certain of the polyoxyethylene emulsifiers can no longer be used in standardized foods.

In order that a chemical may be accepted as a component of foods for which standards have been established, it is necessary that evidence of the harmlessness and innocuousness of the food additive be presented to the Food and Drug Administration, usually at a formal hearing. In some cases, when standardized foods are not involved, this evidence may be presented to the Food and Drug Administration at a private hearing. In the case of standardized foods, the onus of proof must be borne by the manufacturer. The same situation should obtain, it would seem, irrespective of whether or not the food is standardized. However, at present, it is up to the Government to prove that a chemical is deleterious when it is added to a non-standardized food product.

While the nature of the "proof" is not necessarily rigid, and may vary with the type of product involved, there are certain questions which must be answered in all cases, particularly when the substance in question is of an undefined chemical composition. Lehman and co-workers *(10)* have outlined the toxicological studies which they consider necessary when a material is to be added to a food. The most important investigations suggested are outlined in Table 1.

TABLE 1

Technics and procedures suggested in toxicological studies by Lehman et al. *(10)*.

I. Chemistry.
  1. Solubility.
  2. Chemical characterization.
  3. Quantitative detection in micro amounts.

II. Acute Toxicity and Pharmacodynamics.
  1. Oral LD_{50} in several species.
  2. Pharmacodynamics.

III. Allergic Response.
  1. Sensitizing reactions in guinea pigs.

IV. Subacute and Chronic Toxicity.
  1. Subacute toxicity (2 to 4 months' feeding).
    (1) Rats.
    (2) Dogs.
  2. Chronic (long-term) toxicity.
    (1) Rats.
    (2) Dogs.

3. Reproduction studies.
4. Paired-feeding.

V. Biochemical Studies.
1. Absorption, distribution, excretion.
2. Detoxication mechanism and fate.
3. Organ function tests.
4. Studies on enzymes.

VI. Pathology.
1. Gross examination of organs and tissues.
2. Detailed histological examination of organs and tissues of control and experimental animals.

It is evident, from the number of procedures concerning which information about the action of an unknown chemical is requested, that obtaining proof of the harmlessness of a compound suggested for use in foods is not a simple matter. It may not only require several years to amass the evidence but also a considerable outlay of money—and still an unequivocal answer may not be obtained.

Our own laboratories have had several opportunities to obtain information essential for permitting the use of additives in foods. When the product involved is a natural one, normally occurring in foods, the amount of such information required may be minimal. Thus, in obtaining permission from the U. S. Food and Drug Administration to include β-carotene as a source of vitamin A in margarine, and also as a coloring agent for the margarine, it was only necessary to demonstrate that β-carotene added to margarine gave the same provitamin A activity alone and in the presence of preformed vitamin A as is obtained when it is used as a source of vitamin A in the usual bioassay procedures. In fact, a suggestion was made that margarine was an especially favorable vehicle for supplying β-carotene, as there appeared to be some potentiation of the vitamin A-activity when the pigment was administered in this manner to rats *(6)*. Evidence was likewise obtained of the stability of β-carotene in margarine over long periods of storage, as determined both chemically and biologically. In addition, information was provided as to available methods of analysis and concerning the composition of the commercial compound available. The U. S. Food and Drug Administration now permits the use of β-carotene in place of vitamin A in the margarine standard. Because of the intensity of the color, it is only feasible to supply about one-third of the added vitamin A in this form.

Proving the harmlessness of citrates. An example of a study in which a large amount of information was required to prove the harmlessness of the product was the result of the proposed use of isopropyl and stearyl citrates in margarines and fat products. These compounds were found to be especially effective in preventing the so-called flavor reversion of refined soybean oil. There was adequate evidence in the literature of the innocuousness of isopropyl alcohol in the quantities employed *(9, 11, 12)*. Moreover, stearyl alcohol had repeatedly been shown to be a normal product which is synthesized in the intestine *(13, 16)*. Finally, citric acid is a compound widely distributed in our foods, and one normally synthesized continuously from acetate and oxalacetate *(8, 15)*. However, evidence was lacking as to whether or not the citric acid esters of isopropyl alcohol and stearyl alcohol were harmless, since one might suppose that these compounds could not be absorbed and would consequently produce some undesirable effects on other dietary components. Another question was whether, if absorbed unchanged, the ester might exhibit some deleterious effects in the body. It was necessary to follow all of the extensive suggestions made by the Food and Drug Administration. When these several tests were carried out, it was found that there was no evidence of toxicity when isopropyl citrate or stearyl citrate was fed at levels 500 times that at which it would be ingested when added to margarines, to the extent of 0.02% or 0.15%, respectively *(5, 15)*. The U. S. Food and Drug Administration has modified the Standard of Identity of margarine to permit the inclusion of these citrates *(1)*.

Sorbic acid. When the metabolism of a substance is known, or when it can be correlated with a recognized pattern of catabolism, the information necessary to assure oneself of the harmlessness of the product may be considerably abbreviated. This situation occurred in the case of sorbic acid, the use of which as a fungistatic agent

was proposed. Sorbic acid is a 6-carbon dienoic acid ($CH_3 \cdot CH : CH \cdot CH : CH \cdot COOH$) which compares with caproic acid ($CH_3 \cdot (CH_2)_4 \cdot COOH$), normally found in some fats.

As a preliminary test, we did carry out LD_{50} and growth tests with sorbic acid. These results were compared with those obtained when comparable amounts of sodium benzoate were employed. In the first place, sorbic acid is normally metabolized to carbon dioxide and water in the body (with the release of 6.63 Cal. per g.).

$$C_5H_7COOH + 7\ O_2 \longrightarrow 6\ CO_2 + 4\ H_2O + 6.63\ Cal.$$

The normal oxidation of sorbic acid in the animal body.

The LD_{50} values obtained by two independent laboratories were higher for sorbic acid or sodium sorbate than for sodium benzoate, *i.e.*, greater quantities of sorbate could be tolerated than of benzoate. Thus, the LD_{50} of sodium sorbate for Laboratories A and B were 4.0 g./kg. and 5.94 g./kg., respectively, as compared with values of 2.1 g./kg. and 3.45 g./kg. for sodium benzoate. The ratios of LD_{50} (sodium sorbate) : LD_{50} (sodium benzoate) reported by the two laboratories were 1.90 and 1.72, respectively. Sorbic acid was found to have an LD_{50} of 10.50 ± 1.96 g./kg. Table 2 gives a summary of the effects of several levels of sorbate and benzoate on the weight of kidneys and livers of rats. Results were also obtained on the weight gain and hemoglobin of dogs which received sorbate, caproate, or a control diet over a period of 3 months.

The gain in weight of the animals in any one litter on the several dietary regimens was fairly uniform, and the level of hemoglobin at the termination of the tests was satisfactory in all cases (control diet, 11.4%; caproic acid diet, 13.6%; sorbic acid, 12.2%) (3).

The final proof of the harmlessness of sorbic acid was greatly simplified because it was possible to demonstrate that the acid followed the same pattern of metabolism as do naturally-occurring fatty acids. In fact, Witter, Newcomb, and Stotz *(17)* employed sorbic acid in their *in vitro* studies, and stated that "sorbic acid, an unsaturated 6-carbon acid, was used as a model substrate."

When ketogenic acids are fed in appropriate dosages as their sodium salts to fasting rats, a ketonuria of considerable magnitude obtains. The chief end-products excreted in the urine under these conditions are as follows:

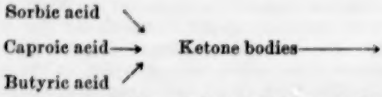
$CH_3 \cdot CHOH \cdot CH_2 \cdot COOH$	$CH_3 \cdot CO \cdot CH_2 \cdot COOH$	$CH_3 \cdot CO \cdot CH_3$
β-Hydroxybutyric acid	Acetoacetic acid	Acetone

The so-called "ketone bodies" which originate from fatty acids and are excreted in the urine during fasting, carbohydrate deprivation, and in some metabolic diseases.

When tests were carried out with female rats given butyrate, caproate, or sorbate, at levels equivalent to 75 mg. (low dose) or 150 mg. (high dose) per 100 sq. cm. of surface area per day in two divided doses, the excretion of ketone bodies was shown to be identical *(4)*. This is excellent evidence that sorbic acid follows the same course of catabolism as do the normally-occurring caproic and butyric acids. During fasting, the end-products would be ketone bodies, carbon dioxide, and water.

Under normal conditions of alimentation, these ketone bodies presumably are oxidized completely to carbon dioxide and water, and yield their full energy capacity. In the presence of minimum quantities of glucose, the decrease in ketonuria is quantitatively similar in the case of butyrate, caproate, and sorbate *(4)*.

These data afford further confirmation of the fact that sorbate follows the pathway of fatty acid metabolism identical with that of caproate and butyrate. These changes are illustrated below.



The oxidation of fatty acids to carbon dioxide and water in the presence of glucose.

Supplementary evidence of the fact that unsaturated fatty acids follow a normal metabolic pattern is evident from the results on crotonic acid as compared with butyric acid. These studies to determine the toxicological properties of sorbic acid have been published in a recent number of *Food Research (3, 4)*.

TABLE 2

The effect of feeding several levels of sorbate and benzoate over a 90-day period on the body weight and liver and kidney weights of rats.

Category	Control tests	Diets containing sorbic acid in %					Diets containing sodium benzoate in %			
		0.5	1.0	2.0	4.0	8.0	1.0	2.0	4.0	8.0
Test substance, g./kg./day	0.0	0.32	0.63	1.26	2.48	5.08	0.64	1.32	2.62	6.29
Diet consumed, g./rat/day	11.97	12.57	12.62	11.98	12.08	12.36	12.69	12.63	12.30	11.98
p for t-test	.....	0.65	0.55	0.99	0.93	0.72	0.54	0.62	0.75	0.99
Av. weight gain, g.	156.3	164.4	169.0	161.8	190.1	158.6	170.7	160.5	150.0	100.2
p for t-test	.....	0.76	0.60	0.84	0.29	0.94	0.58	0.86	0.82	0.06
Liver weight, % body weight	3.17	3.20	3.27	3.25	3.52	3.54*	3.01	2.97	3.12	4.46*
p for t-test	.....	0.83	0.52	0.51	0.06	0.04	0.26	0.26	0.89	0.0005
Kidney weight, % body weight	0.64	0.63	0.64	0.62	0.65	0.65	0.64	0.65	0.70	0.90*
p for t-test	.....	0.65	1.00	0.24	0.73	0.65	1.00	0.65	0.06	0.0001
Uninfected deaths	9	9	9	9	7	7	9	10	8	7
Toxic deaths	0	0	0	0	0	0	0	0	0	4
Sets with any pathology	0	0	0	0	0	0	1	1	1	7

* Results statistically significant when compared with control.

CONCLUSION

In conclusion, it should be pointed out that the proof of innocuousness may be a long drawn out procedure in cases in which little is known of the metabolic fate of the product under study. However, when the catabolism of the proposed additive can be fitted into already well-recognized and well-established patterns of biological reactions, then considerably less information may be required for proving in an unequivocal manner the harmlessness of the product as compared with that which has been considered essential in the past.

LITERATURE CITED

1. Calbert, C. E., Greenberg, S. M., Kryder, G., and Deuel, H. J., Jr. The digestibility of stearyl alcohol, isopropyl citrates, and stearyl citrates, and the effect of these materials on the rate and degree of absorption of margarine fat. *Food Research*, **16**, 294-305 (1951).
2. Delaney, J. J. *Investigation of the Use of Chemicals in Foods and Cosmetics* (82nd Congress, 2nd Session, House of Representatives Report No. 2356, Union Calendar No. 743, Washington, D. C., 39 pp., 1952).
3. Deuel, H. J., Jr., Alfin-Slater, R., Weil, C. S., and Smith, H. F., Jr. Sorbic acid as a fungistatic agent for foods. I. Harmlessness of sorbic acid as a dietary component. *Food Research*, **19**, 1-12 (1954).
4. Deuel, H. J., Jr., Calbert, C. E., Anisfeld, L., McKeehan, H., and Blunden, H. D. Sorbic acid as a fungistatic agent for foods. II. Metabolism of α,β-unsaturated fatty acids with emphasis on sorbic acid. *Food Research*, **19**, 13-19 (1954).
5. Deuel, H. J., Jr., Greenberg, S. M., Calbert, C. E., Baker, R., and Fisher, H. R. Toxicological studies on isopropyl and stearyl citrates. *Food Research*, **16**, 258-280 (1951).
6. Deuel, H. J., Jr., Greenberg, S. M., Savage, E. E., and Melnick, D. The augmentation of the provitamin A potency of carotene when fed in margarine. *J. Nutrition*, **43**, 371-387 (1951).
7. Food Protection Committee, Food and Nutrition Board, *Safe Use of Chemical Additives in Food* (National Research Council, Washington, D. C., 26 pp., 1952).
8. Kalnitsky, G. The formation of citrate by extracts of rabbit kidney cortex. *J. Biol. Chem.*, **179**, 1015-1025 (1949).
9. Lehman, A. J., and Chase, H. F. Acute and chronic toxicity of isopropyl alcohol. *J. Lab. Clin. Med.*, **29**, 561-567 (1944).
10. Lehman, A. J., Lang, E. P., Woodard, G., Draize, J. H., Fitzhugh, O. G., and Nelson, A. A., *Food Drug Cosmetic Law Quart.*, **3**, 412-434 (1949).
11. Lehman, A. J., Schwerma, H., and Rickards, E. Isopropyl alcohol: rate of disappearance from the blood stream of dogs after intravenous and oral administration. *J. Pharmacol. Exptl. Therap.*, **82**, 196-201 (1944).
12. Lehman, A. J., Schwerma, H., and Rickards, E. Isopropyl alcohol. Acquired tolerance in dogs, rate of disappearance from the blood stream in various species, and effects on successive generations of rats. *J. Pharmacol. Exptl. Therap.*, **85**, 61-69 (1945).
13. Schoenheimer, R., and Hilgetag, G. The occurrence and secretion mechanism of cetyl alcohol in the animal organism. *J. Biol. Chem.*, **105**, 73-77 (1934).
14. Smyth, H. F., Jr., Toxicology, *Ann. Rev. Medicine*, **5**, 349-362 (1954).
15. Stern, J. R., and Ochoa, S. Enzymatic synthesis of citric acid by condensation of acetate and oxalacetate. *J. Biol. Chem.*, **179**, 491-492 (1949).
16. Stetten, De W., Jr., and Schoenheimer, R. The biological relations of the higher aliphatic alcohols to fatty acids. *J. Biol. Chem.*, **133**, 347-357 (1940).
17. Witter, R. F., Newcomb, E. H., and Stotz, E. The role of adenosinetriphosphate in the activation of fatty acid oxidation *in vitro*. *J. Biol. Chem.*, **200**, 703-713 (1953).

THE EFFECT OF SWEATING ON KEEPING QUALITY OF SHELL EGGS*

I. L. WILLIAMS AND F. D. YUNG

Departments of Poultry Husbandry and Agricultural Engineering
University of Nebraska College of Agriculture, Lincoln, Nebraska

(Manuscript received July 14, 1954)

Many investigators have reported that washed eggs do not keep as well in storage as unwashed eggs *(5, 6, 9, 10, 11, 13, 17, 18, 19, 20, 23, 29)*. Mallman and Davidson *(22)* reported that cleaning dirty eggs tended to push the organisms on the shell surface into the shell pores, thereby hastening infection of the contents. In their opinion, washing was the most objectionable method of cleaning, since the wet surface gave the micro-organisms an opportunity to enter the egg. Jenkins *(15)* reported that bacteria can penetrate wet shells even though the shells are unbroken. Other workers have shown that washing alone was not detrimental, but that contamination on the shell and in the washing solution was responsible for spoilage *(1, 4, 24, 25, 28, 32)*.

Moisture may condense on the shell surface when eggs are placed in a warmer atmosphere than that in which they have been held. Jenkins, Hepburn, Swan and Sherwood *(16)*, exposed clean and dirty eggs to sweating for a short period of time. No detrimental effects on keeping quality were reported. These workers also subjected eggs after one month's storage to a warm temperature and humid atmosphere for 16 hours. This treatment did not appear to be detrimental to the keeping quality of clean eggs, but the loss in dirty eggs was as great as if they had been washed. Rosser, White, Woodcock and Fletcher *(26)* reported that condensation formed when chilled eggs are brought in contact with warm, moist air, is an important factor in the growth of exterior mold. Forsythe, Ayres and Radlo *(8)* found that after 6 weeks' storage at 25°C., and 50% relative humidity, sweated eggs contained more than twice as many organisms as non-sweated eggs.

The object of this series of experiments was to determine the effect of sweating on keeping quality of fresh laid, clean and soiled eggs.

EXPERIMENTAL

Eggs from the Nebraska Experiment Station S. C. White Leghorn flock were used in this study. These birds are all of similar breeding, housed under uniform conditions, and fed the same ration. For each experiment, the eggs were gathered within a 2-day period. Egg weights ranged from 21-23 ounces per dozen. A series of 7 experiments over a 4-year period was conducted.

Half of the eggs used in Experiments I through IV were artificially soiled by rolling in damp litter on the hen house floor. By soiling these previously clean eggs artificially, it was possible to obtain more uniformity with regard to extent of soiling between the various experiments. Refuse containing chicken feces has been reported

* Published with the approval of the Director as Paper No. 657, Journal Series, Nebraska Agricultural Experiment Station.

TABLE 1

Treatment and storage conditions to which eggs in each experiment were subjected

Experiment No.	Treatment				Storage conditions		
	Group 1		Group 2		Temp. °F.	Relative humidity—%	Time days
	Sweating[1]		Non-sweating[2]				
	Number eggs		Number eggs				
	Clean	Soiled	Clean	Soiled			
I	50	50	50	50	30-34	85-90	180
II	50	50	50	50	30-34	85-90	180
III	50	50	50	50	31-45	60-80	180
IV	50	50	50	50	31-45	60-80	180
V	100		100		75-85	50-60	42
VI	100		100		75-85	50-60	45
VII	100		100		35	80	165

[1] Group 1—Held for 48 hrs. at 40°F.—Sweating induced at end of 48 hrs. by subjecting eggs for 6 hrs. to 80°F. and 50% relative humidity.
[2] Group 2—Held for 48 hrs. at 60°F., and then exposed for 6 hrs. to 80°F. and 50% relative humidity (did not sweat).

by Zagaensky and Lutikova *(32)* and Winter *(31)* to contain micro-organisms responsible for egg spoilage. For each experiment, the eggs were divided into groups and treated as shown in Table 1.

At the conclusion of the 48-hour period, sweating was induced by placing Groups 1 and 2 in a room with a temperature of 80°F. and relative humidity of 50% for 6 hours. On the sweating eggs in Group 1, moisture condensation was profuse, and remained on the shell for 1½ to 2¼ hours. No condensation appeared on the eggs in Group 2.

After exposure to sweating, each group was packed in a 15-dozen size fiber case, and placed in commercial storage. Conditions of temperature and humidity varied as indicated in Table 1.

After storage for 180 days, the eggs (Experiments I-IV) were graded for quality according to broken out appearance. Inedible eggs were not included. The standard used for grading was that described by Van Wagenen and Wilgus *(30)*.

In Experiments V, VI and VII, only clean eggs were used. Egg quality was measured by determining the Haugh Unit *(14)*, with the aid of the Egg Quality Slide Rule, designed by Brant, Otte and Norris *(2)*. Again the inedible eggs were not included.

The number of micro-organisms on the outer shell surface of sweated and non-sweated, clean eggs was determined by washing eggs in a Waring blender following the procedure described by Rosser *(27)*. Aliquots of the wash water were placed in petri dishes covered with nutrient agar, and incubated for 72 hours at 25°C. No effort was made to determine the number of micro-organisms on the surface of sweated and non-sweated soiled eggs.

TABLE 2

Interior quality of sweated and non-sweated eggs after 180 days' storage at 30-34°F., and 80-90% relative humidity.

Experiment No.	Shell condition	Sweating		Non-sweating	
		Quality score	% inedible	Quality score	% inedible
I	Clean	2.95	0	2.54	0
	Soiled	2.91	4	2.54	4
II	Clean	2.56	0	2.54	0
	Soiled	2.74	0	2.54	0

RESULTS AND DISCUSSION

Results of Experiments I and II are shown in Table 2.

Statistical analysis, using Standard Error of the Mean (T-test), as a measure of difference was made. There was no variation in percentage of inedibles between sweated and non-sweated eggs in Experiment I. The test of difference between mean quality scores, however, was significant (5% level) between sweated and non-sweated eggs in both clean and soiled lots. These differences were in favor of the non-sweated eggs.

In Experiment II, variation in mean quality score was not significant between sweated and non-sweated clean eggs. This difference was significant at the 1% level in the soiled lots in favor of the eggs not subjected to sweating.

TABLE 3

Interior quality of sweated and non-sweated eggs after 180 days' storage in fluctuating temperatures (31-45°F.) and humidity (60-80%).

Experiment No.	Shell condition	Sweating		Non-sweating	
		Quality score	% inedible	Quality score	% inedible
III	Clean	4.54	0	4.64	0
	Dirty	3.91	0	4.43	8
IV	Clean	3.94	0	3.90	0
	Dirty	4.14	0	4.18	0

In Experiments III and IV (Table 3), no significant differences were noted in the percentages of inedibles between the sweated and non-sweated eggs in either the clean or soiled lots. There were also no significant differences in the quality scores of the sweated and non-sweated eggs except in the soiled eggs of Experiment III. In this lot, a significant difference (1% level) in favor of the sweated eggs was noted. This result is at variance with those of Experiments I, II and IV, and may be the result of the very unfavorable storage conditions that existed during the course of Experiments III and IV.

In Experiment V (Table 4), there was no significant difference in the quality scores between sweated and non-sweated eggs. The difference in

TABLE 4

Interior quality of sweated and non-sweated clean eggs after storage for various periods of time at room temperature (75-85°F.), and 50-60% relative humidity.

Experiment V				
Length of storage time (days)	Sweating		Non-sweating	
	Quality score (Haugh unit)	% inedible	Quality score (Haugh unit)	% inedible
0	64.58	0	62.41	0
13	38.29	0	39.83	0
20	27.96	0	33.87	0
28	27.91	0	31.45	8.33
35	27.40	16.6	32.73	4.16
42	25.47	20.08	23.00	8.33

TABLE 5

Interior quality of sweated and non-sweated clean eggs after storage for various periods of time at room temperature (75-85°F.), and 50-60% relative humidity.

EXPERIMENT VI

Length of storage time (days)	Sweating		Non-sweating	
	Quality score (Haugh unit)	% inedible	Quality score (Haugh unit)	% inedible
0	70.08	0	71.4	0
15	31.47	0	36.6	0
25	29.2	0	31.0	0
45	23.25	0	32.7	0

TABLE 6

Interior quality of sweated and non-sweated clean eggs stored for various periods of time at 35°F., and 80% relative humidity.

EXPERIMENT VII

Length of storage time (days)	Sweating		Non-sweating	
	Quality score (Haugh unit)	% inedible	Quality score (Haugh unit)	% inedible
0	79.8	0	71.4	0
73	60.16	0	59.43	0
103	59.95	4	57.79	0
165	57.26	0	59.54	4

TABLE 7

Bacterial contamination on the shell surface of sweated and non-sweated eggs twenty-four hours after sweating.

	Sweated	Non-sweated
Surface count	25,000[1,2]	16,650[1,2]

[1] Count on shell surface × 1000.
[2] Average of 12 eggs per treatment.

percentage of inedibles after 28 days was in favor of the sweated eggs, but after 35 and 42 days in storage, it was significant (5% level) in favor of the non-sweated eggs.

In Experiment VI (Table 5), there was no significant difference between inedibles, but the difference in quality scores was significant (5% level) after 45 days' storage in favor of the non-sweated eggs.

No significant differences were observed in either the percentage of inedible or the quality scores in Experiment VII. (Table 6).

Bacterial contamination on the shell surface of sweated and non-sweated eggs 24 hours after exposure is shown in Table 7.

Differences in bacterial population on the shell surface were highly significant. This coincides with the results of Forsythe, Ayres and Radlo *(8)*.

CONCLUSIONS

Differences in keeping quality between clean and dirty shell eggs were small.

These results indicate that sweating is detrimental to keeping quality.

Sweating resulted in a marked increase in the number of micro-organisms on the shell surface.

LITERATURE CITED

1. Bryant, R. L., and Sharp, P. F. Effect of washing on the keeping quality of hen's eggs. *J. Agr. Research*, **48**, 67 (1934).
2. Brant, A. W., Otte, A. W., and Norris, K. H. Recommended standards for scoring and measuring opened egg quality. *Food Technol.*, **5**, 356 (1951).
3. Cole, R. Egg quality; the influence of climate and soil. *U. S. Egg Poultry Mag.*, **42**, 527 (1936).
4. Conner, J. W., Snyder, E. S., and Orr, H. L. The influence of washing and oiling on grade and bacterial content of eggs stored for a nine months' period. *Poultry Sci.*, **32**, 227 (1953).
5. Conner, J. W., Snyder, E. S., and Orr, H. L. The influence of washing and oiling on grade and bacterial content of eggs stored for an eight months' period. *Poultry Sci.*, **31**, 939 (1952).
6. Dawson, L. E., Davidson, J. A., and Sheppard, C. C. The storageability of farm washed eggs. *Poultry Sci.*, **29**, 755 (1950).
7. Erikson, E. What happens to dirty eggs, washed and not washed? Exp. Sta. Record, **53**, 669 (1923).
8. Forsythe, R. H., Ayres, J. C., and Radlo, J. L. Factors affecting the micro-biological populations of shell eggs. *Food Technol.*, **7**, 49 (1953).
9. Funk, E. M. Experiments in Cleaning Soiled Eggs for Storage. University of Missouri Agr. Exp. Sta. Research *Bull. 426* (1948).
10. Gillespie, J. M., Scott, W. J., and Vickery, J. R. Studies in the preservation of shell eggs. II. The incidence of bacterial rotting in unwashed eggs and in eggs washed by hand. *Australian J. Applied Sci.*, **1**, 215 (1950).
11. Gillespie, J. M., Scott, W. J., and Vickery, J. R. Studies in the preservation of shell eggs. III. The storage of machine-washed eggs. *Australian J. of Applied Sci.*, **1**, (3) (1950).
12. Gunderson, M. F. A washed egg can be good. *U. S. Egg Poultry Mag.*, **52**, 111 (1946).
13. Gutteridge, H. S. Washed eggs in storage. *U. S. Egg Poultry Mag.*, **35**, 57 (1929).
14. Haugh, R. R. The Haugh unit for measuring egg quality. *U. S. Egg Poultry Mag.*, **43**, 552 (1937).
15. Jenkins, M. K. Commercial Preservation of Eggs in Cold Storage. U. S. Department of Agriculture, *Bull. 775* (1919).
16. Jenkins, M. K., Hepburn, J. S., Swan, C., and Sherwood, C. M. Effects of cold storage on shell eggs. *Ice and Refrig.*, **128**, 58 (1920).
17. Kahlenberg, O. J., Gorman, J. M., Home, M. A., and Baush, E. K. A Study of the Washing and Storage of Dirty Shell Eggs. U. S. Department of Agriculture *Cir. 911* (1952).
18. Lorenz, F. W., Starr, P. B., and Ogasawara, F. X. The development of pseudomona spoilage in eggs. *Poultry Sci.*, **29**, 769 (1950).
19. Lorenz, F. W., and Starr, P. W. Spoilage of washed eggs. (1). *Poultry Sci.*, **31**, 204 (1952).
20. Lorenz, F. W., Ogasawara, F. X., and Starr, P. B. Spoilage of washed eggs. (2). *Poultry Sci.*, **31**, 221 (1952).
21. Lorenz, F. W., and Almquist, H. J. Seasonal variations in egg quality. *U. S. Egg Poultry Mag.*, **42**, 168 (1936).
22. Mallman, W. L., and Davidson, J. A. Oil protected shell eggs. *U. S. Egg Poultry Mag.*, **50**, 113 (1944).
23. McNally, E. H. Effect of drying after washing on the incidence of eggs infected by spoilage micro-organisms. *Poultry Sci.*, **31**, 1102 (1952).
24. Miller, W. A., and Crawford, L. B. Some factors influencing bacterial penetration of eggs. *Poultry Sci.*, **32**, 303 (1953).
25. Miller, M. W., Joukovsky, V., and Kraght, Aibert. Experiments relating to the spoilage of washed eggs. *Poultry Sci.*, **29**, 27 (1950).
26. Rosser, F. T., White, W. H., Woodcock, A. H., and Fletcher, D. A. Preservation of eggs. (I). Treatments for maintaining quality in shell eggs at ordinary temperatures. *Can. J. Res.*, **20** D, 59 (1942).
27. Rosser, F. T. Preservation of eggs. II. Surface contamination on egg shells in relation to spoilage. *Can. J. Res.*, **20** D, 291 (1942).
28. Sharp, P. F. Dirty and washed eggs. *U. S. Egg Poultry Mag.*, **41**, 28 (1935).
29. Starr, P. B., Lorenz, F. W., and Ogasawara, F. X. Spoilage of washed eggs. (2). *Poultry Sci.*, **31**, 215 (1952).
30. Van Wagenen, A., and Wilgus, H. S. The determination and importance of the condition of the firm albumen in studies of egg white quality. *J. Agr. Res.*, **49**, 51 (1935).
31. Winter, A. R. Black rot in fresh shell eggs. *U. S. Egg Poultry Mag.*, **48**, 506 (1942).
32. Zagaensky, J. S., and Lutikova, P. O. Bacteriological study of the egg. *U. S. Egg Poultry Mag.*, **50**, 121 (1944).

EFFECT OF REDUCTION IN BACTERIAL LOAD DUE TO PREVIOUS EXPOSURE TO HEAT DURING THERMAL PROCESS ON LETHAL RATES AND LETHALITY[a]

H. HURWICZ[b]
The Trane Company, La Crosse, Wisconsin

AND

R. G. TISCHER[c]
Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

(Manuscript received October 2, 1954)

Before an attempt is made to present modified concepts of thermal process evaluation, it is of interest to review in detail the methods used in the past and at the present time. It is also necessary to define the symbols commonly used in such evaluations. These symbols, although commonly used and defined, still cause a certain amount of confusion in the mind of the average reader or food processor attempting to apply them, perhaps because different writers have different views of their meaning and use many subscripts quite liberally and, in some cases, not too consistently.

DEFINITIONS

A clarification of the dimensions of the symbols facilitates greatly their understanding and will be included in their definitions:

U = no. of minutes at any temperature necessary to reduce a load of bacteria to a prescribed level (or according to the original definition, to destroy all bacteria).

F = no. of minutes at 250°F. to achieve the same result as above.

$F_1 = \frac{U}{F} = \frac{\text{no. of min. at any temp.}}{\text{no. of min. at 250°F.}}$ or the number of minutes at any temperature during which the thermal death time curve passes through F = 1.

z = thermal death time "slope" expressed in °F. necessary to pass one log cycle.

F_o = the value of F when z = 18°F.

Z = thermal death rate "slope" expressed in minutes necessary to pass one log cycle or to reduce the population of bacteria by a factor of 10. (Sometimes called D.)

F may be defined alternately as the number of minutes at 250°F. necessary to reduce any initial load of bacteria by a prescribed factor.

All of these variables are connected by the following functional relationships: $U = FF_1 = Z(\log a - \log b)$ where *a* may be the initial concentration of bacteria or their number and *b* the final concentration or number of survivors, and $F_1 = \log^{-1}(250 - T)/z$ or it is equivalent to the number of minutes which would be required at 250°F. to achieve the same effect as holding it at any other temperature for some other length of time ($U/F = F_1/1$); F_1 is then actually a conversion factor permitting evaluation of lethal effects of any temperature in terms of a reference temperature of 250°F.

THE "GENERAL METHOD"

The "general method" of Bigelow (*2*) makes use of graphical integration of thermal data converted to TDT (Thermal Death Time) data by two alternate procedures. In the first one the lethal rates (1/U) are plotted for the corresponding temperatures on

[a] Journal Paper No. J-2612 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1123.

[b] Formerly of Iowa State College, Ames, Iowa.

[c] Formerly of Iowa State College, Ames, Iowa.

the heating curve against time of processing, and in the second, converted lethal rates ($1/F_i$) are plotted against the time of processing and then in both procedures, the areas under the curves are integrated with a planimeter. The second procedure results in a value expressed directly in terms of minutes at 250°F. and the first in a value expressing the number of lethality units which must be multiplied by the F value taken from the TDT curve at 250°F. to give the same results as the alternative procedure.

Both procedures may be represented in tabular form with dimensions of x = minutes at 250°F. and y = minutes at any temperature.

Procedure No. 1

(TDT curve passing through $F \doteq 1 = m$)

STEP 1

Time necessary for destruction at any temperature: $U = FF_i$
Dimensions: y

STEP 2

Lethal rate: $L.R. = 1/FF_i$

Dimensions: $\frac{1}{x(y/x)} = 1/y$

This may be termed the F value per minute at any temperature per unit lethality (or per minute at 250°F.).

STEP 3

Sterilizing value: (L.R.) (time) = $(1/FF_i)$ (t)

Dimensions: $\frac{1}{x(y/x)}$ (y) = 1 = dimensionless

In general t will be a fraction or a multiple of FF_i and thus both F and F_i will cancel out, leaving the result as a dimensionless fraction or multiple of a lethality unit to be multiplied by the F value of TDT curve used. The final value is expressed in minutes at 250°F.

Procedure No. 2

(TDT curve passing through F = 1)

STEP 1

Time necessary for destruction at any temperature adjusted to 250°F. (multiple of minutes at 250°F.): F_i
Dimensions: y/x

STEP 2

Lethal rate: $1/F_i$

Dimensions: $\frac{1}{(y/x)} = x/y$

This may be termed the F value per minute at any temperature.

STEP 3

Sterilizing value: $(1/F_i)$ (t)
Dimensions: (x/y) (y) = x
As in procedure No. 1, t is a fraction or multiple of FF_i, but only F_i cancels out leaving the factor F in the result and expressing it directly in terms of minutes at 250°F.

By means of the above procedures any process at any temperature may be expressed in terms of equivalent time required at 250°F. to achieve the same sterilizing effect as the time at the given temperature. It is assumed that the temperatures are reached instantaneously at the points investigated. These procedures can be adjusted to the concept of thermal death rate by deriving the TDT curves from TDR data for assumed initial and final loads of bacteria, and for the accepted definition of F either as time for reduction by a specific factor, or to a prescribed level.

The relationship between TDR (Thermal Death Rate) data and TDT curves has been discussed in (δ) and z (time) and y = log of survivors (or log of concentrations)

was given in the same reference. xz data were then the TDT data and yz were the TDR data. In general, time is assumed to be an exponential function of the temperature and as such, a straight line results when the log of time is plotted against temperature.

When establishing the lethal rate curves as in these procedures, the reduction in bacterial load at successive temperatures due to the effect of previous exposure to heat is not taken into account. Thus, the time necessary to reduce the decreasing magnitude of bacterial populations at successively increasing temperatures is overestimated as the thermal process goes on. The result is an underestimate of the sterilizing value of the total process. This consideration has not been taken into account up to the present time in process evaluation although it could have a large effect on recommended process times. The investigation of the actual thermobacteriological relationships to be used in ascertaining the sterilizing values of thermal processes would correct the methods of estimate. These remarks are true whether graphical methods of integration or analytical methods are used, since the analytical methods used by Ball (*1*) or Hicks (*3*) assume the slope of TDT curves to be constant. (Ball assumes the relationship of U vs. temperature, and Hicks the relationship of Z vs. temperature. Therefore, for the heating part of the process, the F value estimates are the same.) Hicks defines the F value as the time required for a factor destruction and not to a prescribed level of destruction as Ball does. This is necessitated by the thermal death rate concept. If the Z value is actually a constant for a given temperature, then Hicks' results are correct under his definition of F value. Nevertheless, the Z values are independent of the effect of previous exposure of bacteria to heat and a TDT curve based on them would not be the appropriate one to use. Ball's method based on U values determined for a given initial load of bacteria, on the other hand, disregards the effect of previous exposure of bacteria to heat on the effective initial load for the changing temperatures, and should be corrected for this effect. Therefore in any case, a corrected TDT relationship should be determined. Because of very meager data for the determination of Z (or D) it is not possible to ascertain the true relationship between the number of survivors and time. There is some evidence that Z is not a constant but varies with time and, should this be true, the determination of the actual TDT relationship becomes even more complex and might vary depending on the level of destruction of bacteria desired whether a "by factor" or "to a level" definition of F is accepted.

It seems that for the sake of clarity the equivalent of the time at any temperature necessary to achieve a desired reduction in the load of bacteria as expressed in the number of minutes at 250°F., termed at the present time the F value of the process, should be redefined as F_e (for F equivalent), the symbol F should be reserved in distinction to F_c for the value on TDT curve (for a specific organism assumed to be present at a specified initial concentration) corresponding to the abscissa of 250°F. At the present time these two distinctly different values are expressed by means of the same symbol F.

DEVELOPMENT OF A METHOD OF PROCESS EVALUATION ACCOUNTING FOR CONTINUOUS REDUCTION OF BACTERIAL CONCENTRATION DURING PROCESSING

Methods of evaluation of the sterilizing value (lethality) of a thermal process have been discussed above and mention has been made of the overestimate of time necessary for reduction of the initial bacterial load to a prescribed level when these methods are used. The overestimate results from the assumption that at any time during processing the temperatures are reached instantaneously (with reference to the beginning of the process) whereas in fact the temperatures obtained follow the heating curve. If at zero time the bacterial load is assumed to be (a_1), then at time (t_1), this load has been reduced to (a_2) and at (t_n) to (a_{n+1}) the values of a_2; . . . a_n depend on the temperatures at the point investigated (T_1; . . . T_n) and the length of the exposure to this temperature (t_1; . . . t_n). These variables are connected by the thermal death rate (TDR) relationship:

$$\Delta t_n = t_n - t_{n-1} = Z_n \log \frac{a_n}{a_{n+1}} \quad \text{(Eq. 1a) or}$$

$$t_n = Z_n \log \frac{a_1}{a_{n+1}} \quad \text{(Eq. 1b)}$$

if $t_{n-1} = 0$ and where Z_n is the slope of the TDR curve corresponding to the temperature T_n at the point evaluated.

These equations can be used to determine the time necessary to reduce the bacterial load to a prescribed level if (a_1) and (a_n) are known; if the final load desired (b) is specified, the time necessary for the reduction is:

$$U_n = Z_n \log \frac{a_1}{b}$$

The ratio of t_n/U_n is the fraction of the desired sterilizing effect achieved during the exposure of length t_n. The lethal rate is $1/U_n$. The total sterilizing value of the process is computed by the procedure outlined previously and depends solely on the value of Z_n. The log a_1/b is assumed to be constant, which amounts to the assumption that at any time during processing the same bacterial load (a_1) must be reduced to the final level (b). Under actual processing conditions, however, the bacterial load is continuously decreasing and the bacterial load to be reduced during consecutive intervals of time at constantly increasing temperatures becomes smaller. Thus the time (U_n) necessary to attain a specified sterilizing effect at T_n is affected by the previous exposure to heat at time intervals $\Delta t_{n-1} = t_{n-1} - t_{n-2}$; $\Delta t_{n-2} = t_{n-2} - t_{n-3}$; . . . etc., showing up in decreased bacterial loads to be reduced at consecutive temperatures and assume values (U'_n) smaller than predicted by accepted methods; the new lethal rates ($1/U'_n$) become respectively larger than the ($1/U_n$) presently used.

Referring to the "general method" (*2*) as described previously and to the TDR and TDT relationship presented in Fig. 4 in (*5*) a "modified general method" will be developed to account for the effects of previous exposure to heat when estimating the lethality of a process.

Figure 1 represents the (xy) axis of Figure 4 in reference (*5*) with the log of time plotted against temperature (TDT relationship modified for TDR concept).

The symbols used below have been defined in the introductory section of the paper. The symbols used with a "prime" (′) correspond to the values as used in the proposed method allowing for the previous exposure of bacteria to heat and which are different from the corresponding values used in the conventional methods. These symbols are best understood by inspection of Figure 1 and Table 1. Considering the TDT relationship for each initial load separately the $\frac{1}{U} = \frac{1}{FF_i}$ equality holds whether the old or new concepts are used and therefore $\frac{1}{U'} = \frac{1}{F'F_i}$ and $\frac{F'}{U'} = \frac{1}{F_i}$; and $\frac{U_1}{F_{i_1}} = F_1 = \frac{U'_1}{F_{i_1}} = F'_1$ for the first interval of time, ($n = 1$); however, as it may be observed in Figure 1, for successive intervals of time ($n > 1$)

$$\frac{U_n}{F_{i_n}} = F_1 \pm \frac{U'_n}{F_{i_n}} = F'_n.$$

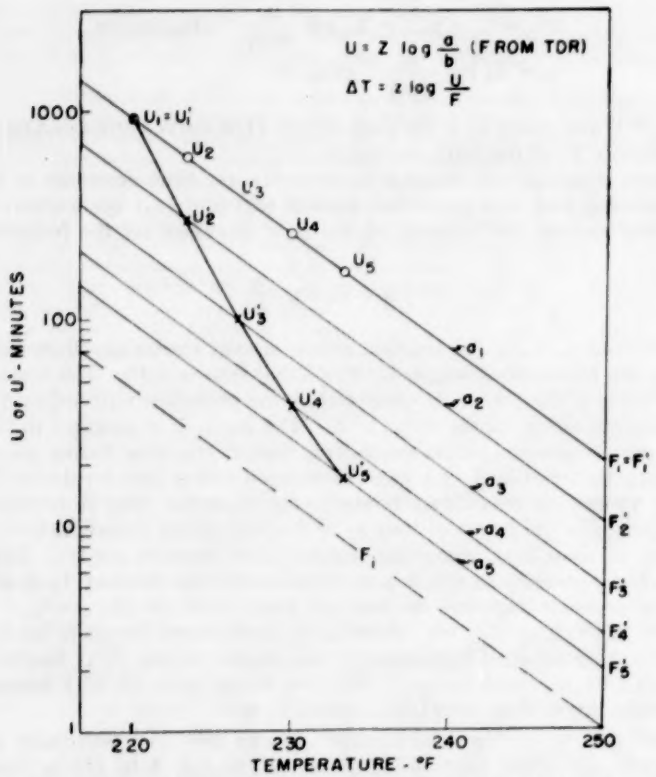


Figure 1. Semi-log plot of a hypothetical TDT relationship resulting from a given thermal process and for varying initial bacterial loads. (a_i)

Thus for every individual time interval considered separately the equivalent value of the process in terms of minutes at 250°F. (F_i) remains the same for both methods, but for the new method

$$\sum_1^n \frac{1}{F_i} \pm F'_1 \sum_1^n \frac{1}{U'}$$

because F'_n is not a constant as was the case for the "general method" where F_1 is a constant and

$$\sum_1^n \frac{1}{F_i} = F_1 \sum \frac{1}{U}$$

gave the total sterilizing effect in terms of minutes at 250°F.; instead, in the new method

$$\sum_1^n \frac{1}{F_i} = \sum_1^n \frac{F'}{U'}$$

TABLE 1
Comparison between two methods of determination of lethality of thermal processes

Proposed Method	"General Method"
STEP 1	
Equal time interval	
$\Delta t_1; \Delta t_2 \ldots \Delta t_n$	$\Delta t_1; \Delta t_2 \ldots \Delta t_n$
STEP 2	
Bacterial load at the end of Δt_n and beginning of Δt_{n+1}	
$a_2; a_3 \ldots a_{n+1}$ $\log a_n + 1 = \log a_n - \Delta t_n/Z_n$ (computed from)	$a_1; a_1 \ldots a_1$ (constant)
STEP 3	
Average temperature during Δt_n	
$T_1; T_2 \ldots T_n$	$T_1; T_2 \ldots T_n$
STEP 4	
Time necessary to reduce initial load (a_1) to the final (b) during Δt_n	
$U'_1; U'_2 \ldots U'_n$ or $Z_1 \log a_1/b; Z_2 \log a_2/b \ldots Z_n \log a_n/b$	$U_1; U_2 \ldots U_n$ $Z_1 \log a_1/b; Z_2 \log a_1/b \ldots Z_n \log a_1/b$
STEP 5	
Lethal rate (reciprocal of U in Step 4)	
$1/U_1; 1/U'_2 \ldots 1/U'_n$	$1/U_1; 1/U_2 \ldots 1/U_n$
STEP 6	
Summation to obtain total number of lethality units	
$\Delta t \sum 1/U' = \Delta t \sum 1/Z \log(a/b)$	$\Delta t \sum 1/U = \Delta t/\log(a_1/b) \sum 1/Z$
(This step may be omitted in either method if lethal rate is plotted against time and then graphically integrated.)	
STEP 7	
Factor U/U'	
$\frac{\log a_1/b}{\log a_1/b}; \frac{\log a_1/b}{\log a_2/b} \ldots \frac{\log a_1/b}{\log a_n/b}$	$1; 1 \ldots 1$

and thus procedure No. 2 cannot be used for evaluation of total sterilizing effect (F_s) in terms of minutes at 250°F. processing temperature. A modified procedure No. 1 is possible of development to result in number of lethality units in a process (fraction or multiple of the specified sterilizing effect in terms of reduction of initial load to a finite final load). This number of lethality units cannot be simply multiplied by the F value of the TDT line to give the F_s value of the process such as:

$$(\text{No. of lethality units}) \times (F) = F_s \qquad \text{Eq. 2a}$$

in the conventional method but instead in the proposed method

$$(\text{No. of lethality units})' \times (F' \text{ effective}) = F_s \qquad \text{Eq. 2b}$$

$$\text{or } (F' \text{ effective}) = \frac{F_s \text{ value (by "general method")}}{(\text{No. of lethality units})' \text{ (by proposed method)}}$$

could be derived as a factor correlating both methods and could serve as a

means of comparison of processes at different temperatures or as a process requirement instead of F. Also from Equations 2a and 2b

$$\frac{\text{F' effective}}{\text{F}} = \frac{\text{(No. of lethality units) by ("general method")}}{\text{(No. of lethality units)' by ("proposed method")}}$$

since (No. of lethality units)' > (No. of lethality units) then F' effective < F which bears out the fact of the underestimate of lethality of a given process according to the conventional method. The factor F' effective, however, would not be constant for all conditions, but would be a function of the slope of the heating curve, and thus depend on the commodity processed, as well as on other processing factors.

The above considerations lead to a new method of estimate of lethal rates during processing, parallel to the procedure No. 1 of the "general method."

The new method is outlined in detail and compared to the "general method" in Table 1.

Steps 1-6 are necessary for the computation of lethality by the new method. If graphical integration is desired, step 6 may be omitted and lethality rates (1/U', step 5) are plotted as in the "general method." Equal time intervals (Δt) are chosen as small as possible for higher precision (4) and the temperature is taken from the heating curve. The number of survivors is computed from the formula given for Δt (Eq. 1a) with the Z value appropriate for the temperature from TDR data. U and U' may be either calculated or taken from the TDT curve appropriate for the bacterial load (a_n) present at the end of one time interval and at the beginning of the next.

To simplify the computations involved, the "general method" may be combined with the new one with the help of a prepared graph (Fig. 2) giving the value of the factor U/U' for different values of the ratios of a_1/b, a_n/b and a_n/a_1 for various initial loads (a_1).

The factor U/U' represents the slope of the line U' = f (U) for various (a_1) values and for a constant b. One may follow the "general method" (steps 1, 3, 4, 5) and then find the factor U/U' either from step 7 or from Figure 2 to obtain the new lethality rate (1/U') = (1/U) (U/U') while step 2 of the proposed method will furnish the necessary information about (a_n) and which follows of (a_n/a_1). The factor U/U' is determined by the intersection of the line of a_n/a_1 with the U/U' line for a given a_1/b ratio; or in other words by following the ordinate of log a_1/b to its intersection with a_n/a_1 line and extrapolating (logarithmically) to obtain the corresponding U/U' line at this intersection. This procedure obviates the necessity of having available TDT lines for all levels of a_n with the exception of a_1 to determine U'_n. It shortens the procedure in computations of U' and is more general because (b) need not be specified or calculated. If (b) has been calculated, log a_n/b may be determined and the factor U/U' found at the intersection of the coordinates of the graph in Figure 2. Workers accustomed to the use of the "general method" may find these two variations of the proposed method easier to follow than is direct calculation of U'.

The graphical integration of lethal rates and the summation of sterilizing values of each time interval as proposed in the new method indicate

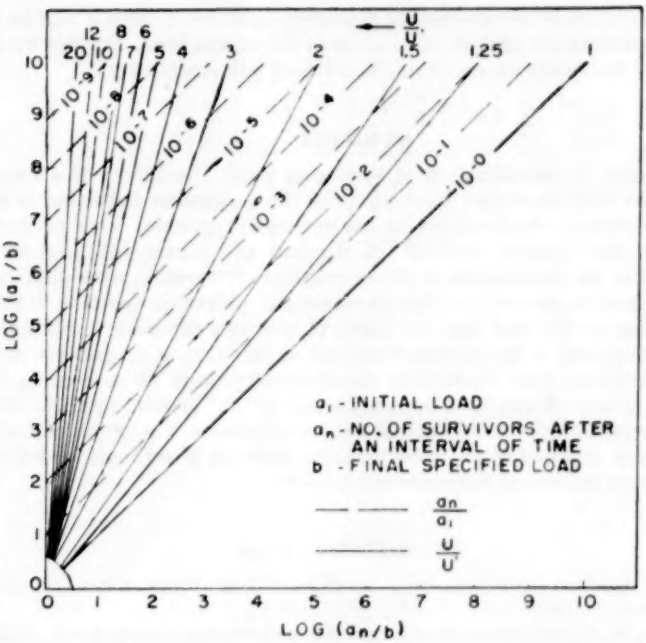

U/U'
12 8 6
20 10 7 5 4 3 2 1.5 1.25 1
10⁻⁹ 10⁻⁸ 10⁻⁷ 10⁻⁶ 10⁻⁵ 10⁻⁴ 10⁻³ 10⁻² 10⁻¹ 10⁻⁰
LOG (a₁/b)
0 1 2 3 4 5 6 7 8 9 10
LOG (aₙ/b)
a₁ - INITIAL LOAD
aₙ - NO. OF SURVIVORS AFTER AN INTERVAL OF TIME
b - FINAL SPECIFIED LOAD
aₙ/a₁
U/U'

Figure 2. Correction factor U/U' for varying ratios of initial bacterial load and number of survivors during pocessing.

that an analytical expression similar to that used in Ball's "Formula Method" *(1)* or by Hicks *(3)* can be derived. The indicated summation for lethality in Table 1, $L = \sum_{1}^{n} \frac{1}{U'\Delta t}$ may be transformed into an integral form $L = \int_{o}^{v} \frac{dt}{U'}$ when $\Delta t \rightarrow 0$. The problem is one of expressing t and U' in terms of temperature (T), or of U' in terms of time (t). The expression of t as $f(T)$ is accomplished from the equations of the heating and cooling curves. Ball expressed U from the TDT data in general form as $U = U_1 e^{\Delta T/z}$. This expression has been modified to account for the logarithmic order of death of bacteria to the form of

$$U = Z \log a/b = Z_1 \log a_1/b\, e^{\Delta T/z}$$

It is necessary now to modify this expression to account for effects of previous exposure of bacteria to heat to obtain a new integral expression discussed above. The new relationship would be of the following general form

$$U' = U'_1 e^{\Delta T/z}.$$

The evaluation of $U' = f(T)$ is a difficult problem. The integral forms which have been derived by the authors are not integrable by ordinary mathematical methods. Taking into account these difficulties it was thought

that the solution of the problem as presented in Table 1, Step 6 will be less complicated than the analytical solution in its present form. Further work is being done to obtain an analytical solution of less complexity.

SUMMARY

A method of determination of sterilizing value (lethality) of a thermal process has been developed to account for the continuous reduction in bacterial population which occurs as the process progresses. This method is similar to the "general method" of Bigelow and others (*4*) used at the present time, as use is made of either graphical integration with planimeter or a summation procedure. The fundamental difference between the two methods lies in the fact that the effect of previous exposure of bacteria to heat is recognized in the proposed method as resulting in progressive reduction of bacterial load exposed to the thermal process at succeeding time intervals. These effects have been neglected in the "general method" which thus underestimated the lethality of a thermal process. The proposed method may be also adapted to the more modern methods of estimate of lethality for the whole volume of the container.

LITERATURE CITED

1. Ball, C. O.; Thermal Process Time for Canned Food. National Research Council *Bull. 37* (1923).
2. Bigelow, W. D., and others; Heat Penetration in Processing Canned Foods. National Canners Association *Bull. 16 L* (1920).
3. Hicks, E. W. On the evaluation of canning process. *Food Technol.*, **5**, 134 (1951).
4. Patashnik, M. A simplified procedure for thermal process evaluation. *Food Technol.*, **7**, 106 (1953).
5. Tischer, R. G., and Hurwicz, H.; Thermal Characteristics of Bacterial Populations. *Food Research*, **19**, 80 (1954).
6. *The Canned Food Reference Manual.* 3rd ed., 1949, American Can Company, New York, N. Y.

APPENDIX

Sample Process Calculation

Reference: The Canned Food Reference Manual (6)

1) TDT curve (Fig. I, p. 304) *cl. botulinum* after Esty & Meyer
2) Heating curve (Fig. II, p. 307)
3) Lethality curve (Fig. III, p. 311)
4) Time-Lethal Heat curve (Fig. IV, p. 312)

Given: $a_1 = 6 \times 10^6$ (*6*, Fig. I, p. 304) — initial load
TDT curve to determine U_a and Z_a (1b1d)

Assume: $b = 6 \times 10^{-3}$ — final load required (this assumption is made only for the purpose of calculation) and amounts to requirement of reduction of concentration by a factor of 10^{12}

Solution: The solution is given below in tabular form following the steps in Table 1 (this paper) compared in summary form with Table III of *The Canned Food Reference Manual*, p. 310.

	Step 1	Step 2		Step 3		Step 4			Step 5		Step 6	Step 7	
n	Δt (min)	a_n General Method	a_n Proposed Method	t Time Proc. (min)	T_a °F Average Temp.	Z_n	U_n Fig. I (6)	U'_n	$1/U_n$	$1/U'_n$		U/U' Cal.	U/U' Fig. 2 Plus Par
1	25–35	6×10^{10}	6×10^{10}	30	211	26.00	416	416	0.0024	0.0024	Summation	1	1
2	35–45	↓	2.93×10^{10}	40	221	3.60	125	122	0.0080	0.0082	of lethal	1.025	1.03
3	45–55		2.67×10^{9}	50	227	4.61	60	53.7	0.0170	0.0186	rates was	1.115	1.12
4	55–65		1.81×10^{7}	60	231	2.46	32	23.4	0.0310	0.0429	performed	1.370	1.37
5	65–75	▼	1.53×10^{5}	70	244	1.61	21	8.7	0.0476	0.1150	graphically	2.415	2.40

To clarify the above results calculations of number of survivors (Step 2) and of ratios necessary for graphical solution based on Fig. 2 of this paper are given below:

n	log a_n(*)	a_n/a_1	log a_n/b
1	$\log 6 \times 10^{10} = 10.7782$ (given)	1.00×10^{0}	$\log (6 \times 10^{10}/6 \times 10^{-3}) = 13.0000$
2	$10.7782 - \frac{10}{26} = 10.4662$	0.488×10^{0}	$\log (2.93 \times 10^{10}/6 \times 10^{-3}) = 12.6884$
3	$10.4662 - \frac{10}{9.6} = 9.4262$	0.445×10^{-1}	$\log (2.67 \times 10^{9}/6 \times 10^{-3}) = 11.6484$
4	$9.4262 - \frac{10}{4.61} = 7.2562$	0.302×10^{-2}	$\log (1.81 \times 10^{7}/6 \times 10^{-3}) = 9.4800$
5	$7.2562 - \frac{10}{2.46} = 3.1862$	0.255×10^{-7}	$\log (1.53 \times 10^{4}/6 \times 10^{-3}) = 5.4065$

* From: $\log a_{n+1} = \log a_n - \frac{\Delta t_n}{s_n}$

Since $U_n = Z_n \log \frac{a_1}{b} = Z_n \log \frac{6 \times 10^{10}}{6 \times 10^{13}}$

$Z_n = U_n/13$

Graphical integration analogical to Fig. 3, p. 311 *(6)* Lethality Curve and Plot of Time — Lethal Heat Curve analogical to Fig. IV p. 312 *(6)* yields required process time (for a reduction by 10^{13} factor) compared to the process time required by General Method (total destruction, which is impossible according to modern concepts) of respectively 64 minutes to 69½ minutes. The reduction in the required process time would be relatively larger for a high temperature process.

Arrows shown on Figure 2 indicate the graphical determination of the U/U^1 factor for a given log a_1/b and a_n/a_1 ratio.

HEAT TRANSFER INTO OPEN METAL THERMORESISTOMETER CUPS[a]

I. J. PFLUG[b] AND W. B. ESSELEN
University of Massachusetts, Amherst, Massachusetts

During the initial testing of the thermoresistometer developed *(6)* in this laboratory for the study of the thermal resistance of bacterial spores it was observed that samples of 0.01 ml. magnitude did not spread themselves uniformly across the bottom of the cup. Instead, a droplet with a depth of more than four times that of a uniform layer was formed either in the center or at the edge of the cup. When larger samples that would cover the bottom of the cup were used, a meniscus was formed at the edge of the cup giving a much greater depth at this point than would be obtained if the sample were spread uniformly across the cup. It was also observed that when sample volumes as large as 0.10 ml. were used the results were not always the same as with smaller samples. Since at high temperatures the time intervals for a series of tests might be in the order of 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 and 0.040 min., a small lag correction factor resulting from increased sample depth could produce erroneous results.

Sognefest and Benjamin *(8)* studied the heating lags in thermal death-time cans and tubes to arrive at accurate correction factors. Stumbo *(9)* calculated the heating rates of samples in his thermoresistometer cup and concluded that since the food samples were within 0.3°F. of the steam temperature for at least 9 seconds (0.15 min.) of a 10 second (0.167 min.) process and for a greater percentage of the time for all longer processes, no correction was necessary. The present study was made to determine the accuracy of exposures as short as 0.005 min. The four phases of the problem presented here are (1) the effect of a lag correction factor on the resulting thermal death-time curve (2) heating characteristics of samples in thermoresistometer cups (3) measurement of the heating rates of samples in thermoresistometer cups, and (4) effect of sample depth on spore destruction time.

The sample cups are 0.443 in. in diameter, 0.333 in. deep, and were drawn from 0.25 lb. tinplate (0.008 in. thick). The depth of sample in the cup unless otherwise stated refers to the depth if the sample were spread uniformly across the bottom of the cup. The initial temperature of the sample is taken at 100.0°F. below the exposure temperature. Lag correction factors are calculated to 0.1°F. below the exposure temperature ($g = 0.1$°F.).

---

[a] Contribution No. 958, Massachusetts Agricultural Experiment Station.

[b] Present address: Agricultural Engineering Department, Michigan State College, East Lansing, Mich.

THE EFFECT OF A LAG CORRECTION FACTOR ON THE THERMAL DEATH-TIME CURVE

A thermal death-time curve that is a straight line on semilogarithmic paper can be described by the equation

$$\log \frac{t}{F} = \frac{250 - T}{z} \tag{1}$$

or

$$t = (F)\ 10^{\frac{(250 - T)}{z}} \tag{2}$$

When data are available for time-temperature relationships that sterilize only a portion of the replicate samples exposed, the method described by Stumbo, Murphy and Cochran *(10)* can be used. The time of heating (U) in minutes at exposure temperature is used in the formula

$$D = \frac{U}{\log a - \log b} \tag{3}$$

to obtain a value "D," which is the time required to reduce the number of organisms by 90%. When a thermal death-time curve is constructed by plotting "D" values as a function of temperature, the resulting curve will be a thermal death-time curve representing the time for 90% (1D) destruction of the organisms. This thermal death-time curve can be described by the equation

$$\log \frac{D}{D_{250}} = \frac{250 - T}{z} \tag{4}$$

Failure to apply a lag correction factor (t_c) may alter a straight thermal death-time curve so that it will no longer be a straight line on semilogarithmic paper. If the lag correction factor is the cause of the digression from the true thermal death-time curve, the digression will take place only when the lag correction factor is significant in comparison to the heating time.

If the heating lag is greater than the cooling lag, the lag correction factor (t_c) will be subtracted from the exposure time (B_b) to give the time of heating (U) or

$$B_b - t_c = U \tag{5}$$

When the cooling lag is greater than the heating lag, the heating time (U) will be the sum of the lag correction factor and exposure time.

When uncorrected values are used; the equation of the thermal death-time curve for 1D is

$$D_f = \frac{B_b}{\log a - \log b} \tag{6}$$

Rearranging equation (5)

$$B_b = U + t_c$$

and substituting in equation (6)

$$D_f = \frac{U + t_c}{\log a - \log b}$$

$$D_f = \frac{U}{\log a - \log b} + \frac{t_c}{\log a - \log b} \tag{7}$$

The error is found by subtracting equation (3) from equation (7)

$$D_f - D = \frac{t_c}{\log a - \log b} \tag{8}$$

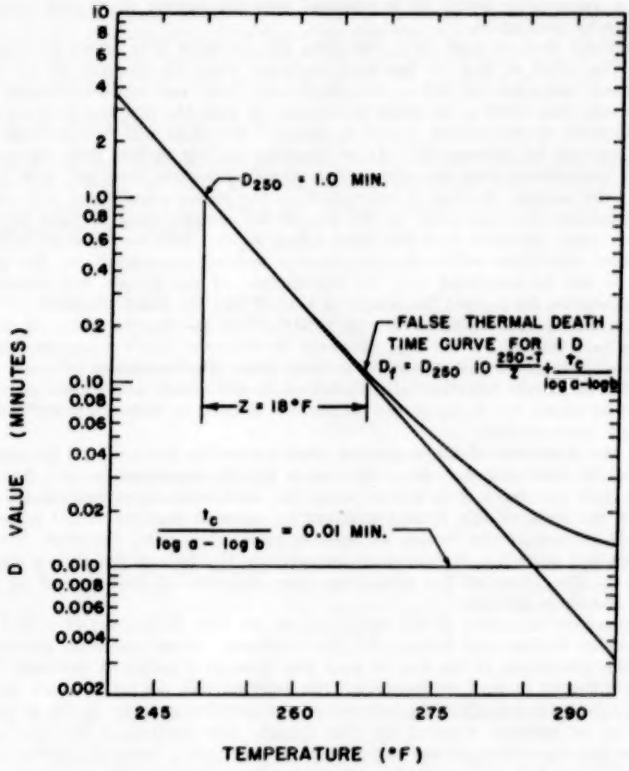


Figure 1. Relationship of true thermal death time curve for 1D, false thermal death time curve for 1D and the D curve error factor $\frac{t_c}{\log a - \log b}$ with a true D_{250} of 1.0 min., z of 18°F. for a 0.10 ml. sample in a thermoresistometer cup where t_c is 0.05 min. and (log a − log b) is 5.0.

In Figure 1 the relationship of D, D_f, and t_c (log a − log b) is illustrated graphically.

When the sample under study fails to reach 0.1°F. below the exposure temperature ($g = 0.1$°F.), the data obtained are not truly representative of the thermal resistance at that temperature and perhaps should be discarded. In the thermoresistometer cup, where the innoculum is assumed to heat as an infinite slab and when the thermal death-time curve has a z (z is defined as the F.° required for the thermal death-time curve when plotted on semilogarithmic paper to traverse one log cycle.) of 18°F. the limiting ratio of B_b/t_c is 2.25. When B_b/t_c is less than 2.25, the point of slowest heating in the sample will not reach 0.1°F. below the exposure temperature.

Heating Characteristics of Samples in Thermoresistometer Cups

The sample of material in a thermoresistometer cup is geometrically a finite cylinder. The height of the cylinder corresponds to the depth of the sample, and the diameter is the inside diameter of the cup. When the sample is introduced into the steam chamber, heat flows from the steam into the sample. The ratio of sample diameter to sample thickness is large enough to permit the sample to be treated as an infinite slab without

introducing appreciable error. It is assumed that the sample is of such consistency that it heats by conduction.

Undoubtedly there is some air surrounding the cup when it is drawn into the steam chamber. The effect of this air has been neglected since the quantity of air is small and the steam molecules can diffuse through the air layer and condense directly on the cup where less than 0.002 g. of steam is required to heat the cup and 0.01 ml. sample.

For purposes of calculation, it will be assumed that heat will flow into the sample from the top and the bottom only. If we consider the top surface first, the sample is at a lower temperature than the surrounding steam; therefore, heat will flow from the steam into the sample. As heat is removed from the steam, some vapor will condense; this condensation will take place on the top of the sample, since initially the sample has a lower vapor pressure than the surrounding steam. This condensation will aid in heat transfer, since there will be mass transfer as well as heat conduction. For practical purposes, it can be concluded that the top surface of the sample will approach the steam temperature the instant the sample is carried into the steam chamber.

In considering the steam metal cup interface on the bottom, some of the same conditions hold true as for the top. However, in this case, any condensate that forms will remain to increase the resistance to heat flow. The coefficient of heat transfer through the steam-cup interface where condensate will slowly accumulate can be estimated within limits *(4)* to be of the magnitude of 2000 to 2600 BTU $ft.^{-2}$ $hr.^{-1}$ $°F.^{-1}$ for filmwise condensation.

Since the resistance of the steam-cup interface is due primarily to the condensate film, it can be concluded that when the cup is dry the resistance to heat flow will be near zero and will reach a maximum when the condensate layer reaches its greatest depth. On the basis of this reasoning it can be assumed that the initial resistance of the outside surface of the bottom of the cup approaches zero; therefore, the bottom of the cup will approach the exposure temperature the instant the cup is surrounded with steam. The effect of the metal cup upon the rate of heating will be greatest when the sample is smallest.

To determine the effect of the metal cup on the rate of heating of a thin sample, the method of Boelter and Tribus *(2)* was employed. Their numerical method makes possible the calculation of the rate of heat flow through 2 bodies of different thickness and with different thermal diffusivities. The problem was set up in work sheet form and then solved numerically. The solution of the problem gave an f_h (f_h is defined as the number of minutes required for the straight line portion of the heating curve plotted on semilogarithmic paper to traverse one log cycle.) value of 0.00036 min. with a j of 1.27 for a 0.01 ml. sample in a thermoresistometer cup. The combined effect of increased f_h value and j would increase the lag correction (t_c) by less than 6%. The result is significant in pointing out that with very thin samples the effect of the cup cannot be disregarded. When the possible variations in sample depth are considered, the over-all effect of the cup in this case is negligible.

It can be concluded at this point that for sample volumes of 0.01 ml. and greater, the error will be small if we consider that the top surface of the sample and the bottom surface of the sample approach the exposure temperature the instant the samples are drawn into the exposure chamber. With these initial conditions, the equation for the f_h value of an infinite slab was found to be

$$f_h = 0.933 \frac{S^2}{a} \tag{9}$$

with $j = 1.273$. These values are given by Olson and Jackson *(5)*. The assumptions that are made in deriving this equation are: that the time intervals are large enough that all terms except the first of the originally derived equation are small enough to be neglected; that the initial temperature distribution through the sample is uniform; and that the surface temperatures are instantly brought up to the final temperature at time zero. Examination of equation (9) shows that the f_h value is directly proportional to the square of the sample half thickness (S).

The f_h value as given by equation (9) is a function of thermal diffusivity and sample depth. Thermal diffusivity is a property of the material being heated and is a function of the temperature; Jackson *(3)* used the value 0.016 $in.^2$ $min.^{-1}$ for food

products that were heated to 240°F. For food products heated to 212°F. the value of 0.015 in.^{2} min.$^{-1}$ is reasonable. The lag correction factor (t_c) that must be evaluated is the difference between the total heating time B_h and the lethality (U) at the exposure or retort temperature. The lag correction factor can be shown in equation form as:

$$t_c = B_h - U \tag{10}$$

Applying the methods of Ball *(1)* where

$$B_h = f_h \ (\log jI - \log g) \tag{11}$$

and making the assumptions that the slope of the thermal death-time curve of the organism under study is 18°F. and that the lag correction factor will be calculated only to a temperature of 0.1°F. below the exposure temperature ($g = 0.1$°F.) the value of the parameter $\frac{fh}{U}$ is 0.58 or

$$U = 1.725 \ f_h. \tag{12}$$

Combining equations (10), (11), and (12)

$$t_c = f_h \ (\log jI - \log 0.1) - 1.725 \ f_h$$

$$t_c = f_h \ (\log jI - 0.725). \tag{13}$$

If, for the general case, the initial temperature is assumed to be 100°F. below the exposure temperature and a value of j of 1.273 is used; the expression for the lag correction factor becomes

$$t_c = 1.379 \ f_h. \tag{14}$$

An expression for the f_h of an infinite slab of finite thickness (28) has been presented as a function of thickness and thermal diffusivity (a) in equation (9). This value can now be substituted in equation (14).

$$t_c = 1.379 \ (0.933 \ S^2/a)$$

$$t_c = 1.285 \ S^2/a \tag{15}$$

Substituting the value for the thermal diffusivity (a) of food products in this temperature range (0.016 in.^{2} min.$^{-1}$); the expression for the lag correction factor (t_c) of a food sample heating by conduction as an infinite slab of finite thickness is given by

$$t_c = 80.4 \ S^2 \tag{16}$$

Measurement of Heating Rates

When the lag correction factor for a thermoresistometer cup is calculated by equation (16), certain errors are present that arise from approximations necessary for solving the problem. An experiment was set up to determine whether the actual heating rates differ markedly from those calculated from theoretical considerations. In the initial tests sample depths of ¼, ⅜ and ½ in. were used. The cups had diameter to depth ratios of 8 and were considered to heat as infinite slabs. The cups were made by soldering a tinplate bottom to sections of 2-, 3-, and 4-in. pipe, respectively ¼, ⅜ and ½ in. long. A thermocouple junction was made from No. 30 gauge wire and was located at the midpoint of the cup.

A 5% bentonite solution, prepared according to the method of Townsend *et al. (11)*, was used as a sample to ensure conduction heating. The cups were heated in steam in a manner similar to the thermoresistometer cups. The steam in these tests had to be at atmospheric pressure to facilitate the rapid introduction of the samples into the steam. An autoclave with the door partly blocked to make a steam reservoir was used as the heating chamber (Figure 2). Temperature control was not a problem since the reservoir of steam was maintained at atmospheric pressure. A thermocouple was located at the center of the sample, and the temperature was measured by a recording potentiometer. Since the results from these tests could not be applied directly to the sample in a thermoresistometer cup, an effort was made to reduce the sample depth to approach more nearly that of a sample in a thermoresistometer cup.

A special steam chamber and cup holding apparatus was constructed (Figure 3) to expose a thin sample in a small (¾ × 1¼ in.) rectangular cup to steam at atmospheric pressure. Filter paper squares the same size as the cup were placed in the cup, a 36-gauge thermocouple was placed between the layers of filter paper so as to be located near the center of the sample. It was not necessary, as pointed out by Olson and Jackson *(5)*, to locate the thermocouple at the exact center of the sample since the slope of the heating curve is a constant for a given sample depth and product. Instru-

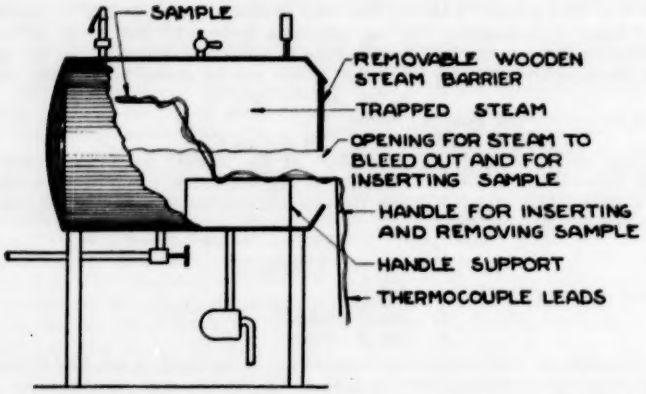


Figure 2. Autoclave with door partially blocked providing steam heating conditions at atmospheric pressure.

mentation was set up to measure accurately the time required for the temperature of the sample to go from 140°F. to 207°F.

The procedure followed in making a test was to pipette a volume of sample into the cup to give the desired depth. Both cup and sample were maintained below 80°F. during the loading period. To expose the sample the circular cup-carrier was turned, carrying the cup and sample into the steam chamber. The time required for the sample to heat from 140°F. to 207°F. was automatically recorded; the exposure temperature was also measured. The f_h value was determined by constructing the portion of the heating curve from 140°F. to 207°F. on semilogarithmic paper according to the method of Olsen and Jackson and then determining the f_h value. It was assumed that this portion of the heating curve was a straight line since the samples in the ¼, ⅜, and ½ in. cups (described above) when heated from 80°F. to 212°F. produced heating curves

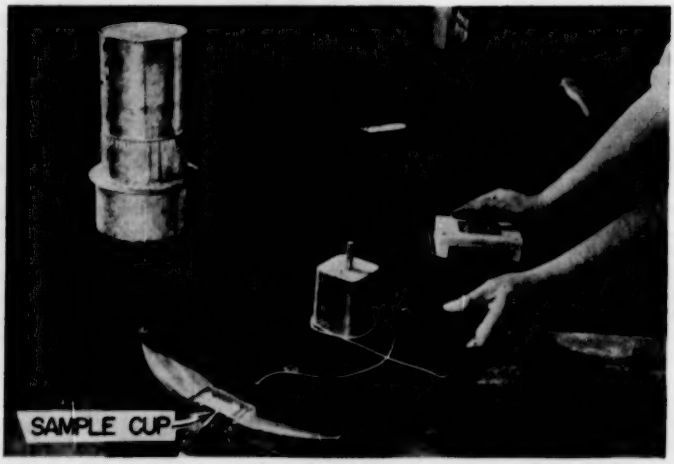


Figure 3. Apparatus for exposing cup with thin samples of media to live steam at atmospheric pressure.

whose straight line portion extended from below 100°F. to 211°F. By this method f_h values were obtained for 3 different sample volumes; the sample depths of these tests were 0.041 in., 0.070 in. and 0.100 in.

The results of the 6 tests are listed below:

Sample depth (inches)	f_h (minutes)
0.041	0.0358
0.070	0.0729
0.100	0.1565
0.250	0.972
0.375	2.15
0.500	3.85

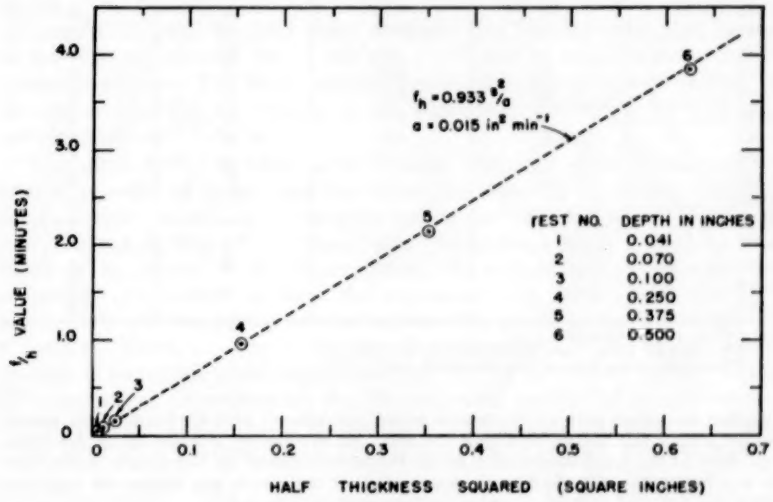


Figure 4. Measured f_h values of media in cups similar to thermoresistometer cups compared to calculated f_h values.

In Figure 4 the results are illustrated graphically. The f_h values as determined experimentally were for all practical purposes the same as those calculated by using equation (9). The thinnest sample we were able to test was 0.041 in. thick and corresponded to an 0.08 ml. sample in a thermoresistometer cup.

Effect of Sample Depth on Spore Destruction Time

The effect of sample depth on spore destruction time was measured to obtain lag correction factor data under actual operating conditions. At 280°F., 24 samples were exposed to a series of time intervals using 0.01 ml. per cup, and repeated using 0.05 and 0.10 ml. per cup. The sample volumes of 0.01, 0.05 and 0.10 ml. gave sample depths of 0.005 in., 0.025 in. and 0.050 in., respectively. Spores of PA 3679 were suspended in neutral phosphate buffer; dilutions were made to give 10,000 spores per cup in each test. The bacteriological procedure was the same as that used by Pflug and Esselen *(7)*. The whole test outlined above was repeated 4 times; the results of one test are presented in Table 1.

The effect of the physical size of the sample was considered in evalauting the variation in results. It was found that a 0.01 ml. droplet would remain in the cup if the cup were inverted. Sometimes the 0.05 ml. sample would remain in the cup when

TABLE 1

Time (minutes)	0.01 ml. per cup		0.05 ml. per cup	0.10 ml. per cup
	Number of positive tubes out of 24 rep.	Range of probability limits at 5% level[1]	Number of positive tubes out of 24 rep.	Number of positive tubes out of 24 rep.
0.060	24	21-24	24	24
0.080	24	21-24	24	24
0.100	24	21-24	24	24
0.120	21	16-23	24[2]	24[2]
0.140	19	14-22	24[2]	19
0.160	23	19-24	18[2]	13[3]
0.180	17	12-21	12	13
0.200	16	11-20	10[3]	9[3]
0.221	11	6-16	6	14
0.241	15	10-20	5[3]	10
0.261	1	0-5	3	7
0.281	2	0-6	4	0
0.301	0	0-3	1	2
0.321	0	0-3	1	2
0.341	0	0-3	1	1
0.359			1	2
0.380			0	0
0.400			0	0
0.420			0	0
0.440			0	0
0.459			0	0
0.478				0
0.500				0

[1] Pflug and Esselen (7).
[2] Exceeds the higher limit of 5% probability level.
[3] Below the lower limit of 5% probability level.

inverted but often part of the sample would fall out. A part of the 0.10 ml. sample would always fall out on inverting the cup. An experiment was set up to determine the effect of the rapid deceleration of the thermoresistometer on the sample in the cups. It was found that with the 0.10 ml. sample some of the sample was thrown out regularly. With the 0.01 and 0.05 ml. samples, no loss could be detected.

RESULTS AND DISCUSSION

Thermal resistance studies at high temperatures and correspondingly shorter heating times are subject to greater potential errors than studies at lower temperatures. Data obtained by any thermal resistance method at the highest temperature possible for the method will be subject to the greatest errors. The point on the thermal death-time curve representing this data will be at the end of the curve. If a heating lag is present and the data are not corrected for this error, a straight line thermal death-time curve will no longer be a straight line (Figure 1). A cooling lag will bend the thermal death-time curve in the opposite direction as a heating lag.

A study of the heat transfer characteristics of a sample in a thermoresistometer cup has led to the conclusions that: (a) Only with a very thin sample will the lag correction factor be negligible for heating times of 0.005 min. (b) When the sample is an infinite slab of finite thickness (2S) the lag correction factor (t_c) will be a function of the square of the thickness. (c) The theoretical lag correction factor (t_c) for an infinite slab of finite

thickness (2S) measured to $g = 0.1°F.$ with $z = 18°F.$ and $a = 0.016$ in.^{2} min.$^{-1}$ is,

$$t_c = 80.4\,S^2 \tag{16}$$

The theoretical heating rates of open metal cups were compared to the measured heating rate for 6 different depths, the thinnest of which corresponds to 0.08 ml. in a thermoresistometer cup. Good agreement was found between the calculated and measured values (Figure 4).

Results from the test measuring the effect of sample depth on spore destruction times fail to point out any significant difference between the 0.10, 0.05, and 0.01 ml. per cup samples. Since the calculated lag correction factor of the 0.10 sample is one hundred times that of the 0.01 ml. sample, the results can only be interpreted to mean that the lag correction factor of the 0.10 ml. samples tested was not significant in relation to the other variables present. The important conclusion that can be drawn is that if the lag of the 0.10 ml. sample is not significant the lag of an 0.01 ml. sample must approach zero.

The effect of the meniscus and of slight tilting of the cup was considered as possibly increasing the lag correction factor in the larger samples. In the 0.01 ml. sample with one filter paper disc the level of the sample is near the top surface of the paper disc. Since the sample is held by the capillary attraction of the paper fibers, the sample will be of uniform thickness regardless of whether the cup is level or tilted. With the 0.05 and 0.10 samples the liquid surface is above the paper disc and a meniscus is formed. When the cup is slightly tilted the depth of the sample near the wall is increased, which should increase the lag correction factor. These phenomena may be responsible for the increased number of stragglers observed with the 0.05 and 0.10 ml. samples. An attempt was made to relate tilted cups with positive tubes, but no significant results were obtained.

SUMMARY

A study of the heating rates and lag correction factors of samples in thermoresistometer cups has been made. An equation was developed for the lag correction factor of a sample in a thermoresistometer cup as a function of sample depth. The heating rates of samples in cups that were larger but had heating characteristics similar to samples in thermoresistometer cups were determined and found in agreement with calculated values. It was impossible to measure the heating rate of a sample thinner than the equivalent of an 0.08 ml. sample in the thermoresistometer cup. A study was made on spore destruction times when the same number of spores were used as an 0.01, 0.05 and 0.10 ml. sample per cup. This study, which was repeated four times, gave added proof that the lag correction factor of an 0.01 ml. sample in a thermoresistometer cup is insignificant and can be neglected.

Acknowledgment

Acknowledgment is due Dr. Carl S. Roys of the School of Engineering for his generous cooperation and assistance in certain phases of this investigation.

LITERATURE CITED

1. Ball, C. O. Mathematical solution of problems on thermal processing of canned food. Univ. of Calif. *Pubs. in Public Health*, 1, (1928).
2. Boelter, L. M. K., and Tribus, M. Numerical solutions for thermal systems. In *Numerical Methods of Analysis in Engineering*. 1949, The Macmillan Co., New York, N. Y.
3. Jackson, J. M. Mechanisms of heat transfer in canned foods during thermal processing. *Proceedings, Institute of Food Technologists*. (1940).
4. Jacob, M. *Heat Transfer*. 1949, John Wiley and Sons, New York, N. Y.
5. Olson, F. C. W., and Jackson, J. M. Heating curves, theory and practical application. *Ind. Eng. Chem.*, 34, 337 (1942).
6. Pflug, I. J., and Esselen, W. B. Development and application of an apparatus for study of thermal resistance of bacterial spores and thiamine at temperatures above 250°F. *Food Technol.*, 7, 237 (1953).
7. Pflug, I. J., and Esselen, W. B. Observations on the thermal resistance of putrefactive anaerobe No. 3679 spores in the temperature range of 250-300°F. *Food Research*, 19, 92 (1954).
8. Sognefest, P., and Benjamin, H. A. Heating lag in thermal death time cans and tubes. *Food Research*, 9, 234 (1944).
9. Stumbo, C. R. A technique for studying resistance of bacterial spores to temperatures in the higher range. *Food Technol.*, 2, 228 (1948).
10. Stumbo. C. R., Murphy, J. R., and Cochran, J. Nature of thermal death time curves for P. A. 3679 and *Clostridium botulinum*. *Food Technol.*, 4, 321 (1950).
11. Townsend, C. T., Reed, J. M., McConnell, J., Powers, M. J., Esselen, W. B., Somers, I. I., Dwyer, J. J., and Ball, C. O. Comparative heat penetration studies on jars and cans. *Food Technol.*, 3, 213 (1949).

REDUCED AND TOTAL ASCORBIC ACID VALUES OF THIRTY-FOUR FOODS[a, b, c]

KATHERINE H. FISHER AND MARY L. DODDS

College of Home Economics, The Pennsylvania State University

(Manuscript received November 26, 1954)

It is known that both the reduced and the dehydroascorbic acid of foods are utilized by the human subject *(3, 4, 5, 6)*. Many of the reported ascorbic acid values of foods are based upon the reduced form of the vitamin *(1)*. Values have been reported for the reduced ascorbic acid content of canned foods *(2)*, foods served in institutional quantities *(9)* and many other processed foods, but the total ascorbic acid values (reduced plus dehydro) of many of these foods are not available. Furthermore, only few of these abundant data for reduced ascorbic acid are for foods as served. In this laboratory, foods which were used in human nutrition studies have been assayed by chemical methods for reduced and for total ascorbic acid. The purpose of the present report is to make these ascorbic acid values available to others.

EXPERIMENTAL

Thirty-four foods, including fruits, vegetables, meats, and dairy products, have been assayed for reduced and for total ascorbic acid. The number of samples of the respective foods examined ranged from 5 to 79.

Although the same brands of commercially canned foods were used throughout the nutritional studies, the vitamin values reported are for different samples owing to the duration of the studies—3 years. At the beginning of each experimental year, new lots of canned foods were purchased. The dairy products were supplied by the University Creamery. Ground beef was purchased in large amounts, packaged in 100 g. portions and frozen.

The foods were sampled for ascorbic acid assay before meals as they were served. The canned vegetables were water-packed and the canned fruits were processed in heavy syrup. Freshly opened canned foods were used. The foods were drained and only the solid portions served and sampled. The amount of sample used for analyses varied from 25 to 50 g. depending upon the ascorbic acid content. One per cent oxalic acid was used to stabilize the ascorbic acid in the fruits and vegetables while a 3% metaphosphoric acid was used to stabilize the ascorbic acid in the meats and dairy products. A Waring blender was used to prepare the slurry and the acidic filtrate was analysed for reduced ascorbic acid by the procedure of Loeffler and Ponting *(7)* and for total ascorbic acid by the method of Roe and Oesterling *(8)*. This latter procedure does not take into account diketogulonic acid if present. The dry weights of the foods were determined.

RESULTS AND DISCUSSION

The average values (and their standard deviations) of the reduced and of the total ascorbic acid content of 34 foods are presented in Table 1.

---

[a] Paper *1911, Journal Series,* Pennsylvania Agricultural Experiment Station, authorized for publication on *September 30, 1954.*

[b] Research Contribution No. 126, College of Home Economics.

[c] This research was supported in part by the United States Department of Agriculture through a contract sponsored by the Human Nutrition Branch of the Agricultural Research Service.

TABLE 1

Average values and standard deviations of reduced and total ascorbic acid of 34 foods

Food	Process	Ascorbic Acid				
		No. samples reduced		S.D.	Total	S.D.
		Analyzed	mg/100 g.	±	mg/100 g.	±
Fruits						
applesauce	Canned, sweetened	19	0.5	0.43	1.6	1.14
apricots	Canned, halves	19	3.5	1.46	4.4	1.94
apricot juice	Canned	17	1.9	0.83	2.5	0.66
cherries (Royal Anne)	Canned	22	3.3	0.68	4.1	1.77
fruit cocktail	Canned	11	1.5	0.72	2.8	1.84
peaches	Canned, halves	18	2.7	1.37	3.4	1.44
peaches	Fresh	10	5.8	1.74	8.7	2.16
pears	Canned, halves	18	0.6	0.45	1.7	0.86
pineapple	Canned, rings	8	9.3	2.82	8.8	4.09
pineapple juice	Canned	20	7.0	1.60	6.9	1.62
plums (purple)	Canned, halves	11	1.5	1.23	1.8	1.68
orange juice	Canned	9	42.6	5.45	52.7	12.93
Meat, Fish and Poultry						
beef	Ground, broiled	44	1.1	0.51	1.2	0.20
beef	Roasted	14	1.1	0.91	1.4	0.78
chicken	Fried	7	0.5	0.45	1.6	0.78
fish (Haddock)	Fried	10	0.9	0.62	1.9	1.48
pork	Roasted	13	0.9	0.68	1.1	0.57
Milk Products						
ice cream	Vanilla	10	0.5	0.51	0.9	0.91
milk	Homogenized, pasteurized	79	0.4	0.34	1.2	1.00
Vegetables						
asparagus	Frozen[2]	11	8.1	1.03	35.0	2.41
beans, green	Canned, cut[1]	41	2.6	1.35	2.6	1.48
beans, green	Fresh, cut[2]	10	14.3	2.22	17.5	4.13
beans, Lima	Frozen[2]	10	10.8	1.14	15.6	2.36
Brussels sprouts	Frozen[2]	10	61.6	5.00	65.6	8.99
carrots	Canned, diced[1]	26	0.7	0.42	0.8	0.42
carrots	Raw, sticks	9	4.2	0.80	4.3	1.14
celery	Raw, sticks	5	4.2	1.02	4.9	2.10
corn	Canned, whole kernel[1]	8	3.0	1.54	3.8	2.11
lettuce, Iceberg	Raw	8	0.9	0.77	2.3	1.33
peas	Canned[1]	18	8.4	3.65	9.4	2.61
peas	Frozen[2]	11	14.3	1.14	19.8	1.39
peas and carrots	Canned (1:1)[1]	8	4.5	1.34	4.7	1.07
relish, pickle	Canned	6	0.3	0.03	1.9	0.21
tomato juice	Canned	7	16.3	4.62	20.6	7.93

[1] Heated to full boil in uncovered pan.

[2] Added to boiling water and cooked 4 minutes after return to boil in uncovered pan.

[3] Added to boiling water and cooked 10 minutes after return to boil in uncovered pan.

For the majority of the foods, the total ascorbic acid values were slightly but consistently higher than the reduced ascorbic acid values. Frozen asparagus and canned orange juice exhibited marked differences in the reduced and total vitamin content. The asparagus exhibited the greatest average difference, 8.1 mg. per 100 g. of the reduced form and 35.0 mg. per 100 g. of total ascorbic acid. Values of 42.6 mg. per 100 g. and 52.7 mg. per 100 g., reduced and total ascorbic acid respectively, were found for the

orange juice. Canned pineapple and pineapple juice exhibited reduced ascorbic acid values slightly higher than but not significantly different from the total ascorbic acid values. Mention is made of it because this has been encountered in this laboratory in connection with the measurement of urinary ascorbic acid. The reason for this anomaly is not known.

The results of 79 analyses of homogenized, pasteurized milk, made during the fall, winter, and spring indicate ascorbic acid values of 0.4 mg. per 100 g. (reduced) and 1.2 mg. per 100 g. (total) or 3.9 mg. per quart (reduced) and 11.7 mg. per quart (total). While milk is not usually considered an important source of ascorbic acid these data show that, on the average, significant amounts of the vitamin may be obtained if one quart of pasteurized, homogenized milk is included in the daily food intake.

SUMMARY

Thirty-four foods, including fruits, vegetables, meats, and dairy products, have been analyzed for reduced and for total ascorbic acid. For the majority of these foods, the total values were slightly but consistently higher than the reduced ascorbic acid values. Marked differences in the amounts of the reduced form and of the total ascorbic acid were found in frozen asparagus and canned orange juice. The total ascorbic acid values for homogenized, pasteurized milk indicate that one quart of milk may supply a significant amount of ascorbic acid.

LITERATURE CITED

1. Agricultural Research Service, Human Nutrition Research Branch. Composition of foods—raw, processed, prepared. U. S. Dept. of Agriculture, Agr. Handbook 8, 8 (1950).
2. American Can Company Research Division. *The Canned Food Reference Manual.* 3rd ed. (1949).
3. Chen, D., and Schuck, C. Human utilization of ascorbic acid in some fruits and vegetables. IV. Potato, tomato, blackberries, cantaloupe, Swiss chard, green pepper, cauliflower, and black raspberries. (In press.)
4. Davey, B. L. Human utilization of ascorbic acid in some fruits and vegetables. II. Strawberries, tangerines, broccoli, cabbage (raw and cooked), collards, kale, sweet potatoes, and turnip greens. (In press.)
5. Davey, B. L., Dodds, M. L., Fisher, K. H., Schuck, C., and Chen, S. D. Human utilization of ascorbic acid in some fruits and vegetables. I. Plan of study and ascorbic acid content of the foods. (In press.)
6. Fisher, K. H., and Dodds, M. L. Human utilization of ascorbic acid in some fruits and vegetables. III. Tomato juice, green beans, Lima beans, Brussels sprouts, orange juice, green peas, asparagus, and peaches. (In press.)
7. Loeffler, A. J., and Ponting, J. D. Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. *Ind. Eng. Chem., Anal. Ed.*, 14, 846 (1942).
8. Roe, J. H., and Oesterling, J. J. The determination of dehydro-ascorbic and ascorbic acid in plant tissues by the 2,4-dinitrophenylhydrazine method. *J. Biol. Chem.*, 152, 511 (1944).
9. Storvick, C. A., Davey, B. L., Nitchals, R. M., and Coffey, R. E. Reduced ascorbic acid content of foods served in institutional quantities. *Food Research*, 15, 373 (1950).

THE RESPIRATORY ENZYMES OF MEAT
I. IDENTIFICATION OF THE ACTIVE ENZYMES

NORMAN H. GRANT
Research Division, Armour and Company, Chicago, Illinois

(Manuscript received December 2, 1954)

The aim of this study was a systematic survey of the surviving respiratory enzymes of meat. Three levels of identification were sought. First was identification of a working enzyme system, complete with coenzymes and substrate. The second was evidence for the occurrence of the enzyme and coenzymes but not necessarily optimum amount of substrates. Third was evidence for the likelihood of merely potential enzymatic action.

Of the numerous changes which meat undergoes, perhaps the most far-reaching are those which relate to oxygen. The interaction of oxygen and meat is mediated by the external supply of the gas, the abundance of myoglobin, and the activity of the surviving muscle respiratory apparatus.

In the living animal, the external oxygen is provided by the circulating blood and limited only by the degree of muscle vascularization. In meat, the external oxygen supply depends upon factors such as wrappings and the juxtaposition of other meat. In the living animal, the concentrations of myoglobin and at least one respiratory enzyme system appear to be related to each other and to the extent of muscular exertion *(7)*. Meat, regarded simply as excised muscle, initially has the same level of enzymes as the functioning muscle. With the passage of time, however, irreversible changes occur. Thermodynamic instability, perhaps buttressed by cathepsin-activated hydrolysis, characterizes proteins in excised tissue.

Although the trend toward degradation is inescapable, the rate varies widely among different enzymes under the same conditions of tissue storage. The oxygen consuming roles of the less labile enzymes will contribute to the state of the myoglobin. The metabolic formation of hydrogen peroxide, without immediate destruction, leads to metmyoglobin formation *(8)*. The mere lowering of oxygen tension, without peroxides, leads to the same result *(4, 6, 14)*.

In the presence of surplus oxygen, myoglobin of fresh meat is in the ferrous, oxygenated, and bright red state. In the presence of a curtailed oxygen supply, much of the myoglobin is in the ferric, non-oxygenated, and brown state. In the absence of oxygen, the pigment is in the ferrous, non-oxygenated, and purple-red state. Any given enzyme, X, might be: (a) inactive, making no demands on oxygen; (b) one link in a chain of operating enzymes, making limited demands on oxygen; or (c) the sole active enzyme, draining off the bulk of oxygen stored by myoglobin. The incorporation of an inhibitor of X in the center of a packed loaf of ground fresh beef would, therefore, pinpoint X as the significant enzyme if the interior bright red color were preserved. A change to brown metmyoglobin would point to X plus other enzymes or indicate a weak inhibitor. A change to purple, as in the controls, would eliminate X. This formed the basis of the inhibitor method of identification.

The other two criteria of identification were the extent of methylene blue reduction and the rate of oxygen consumption.

EXPERIMENTAL PROCEDURE

The method of inhibitors. Beef trimmings were ground, thoroughly mixed with the additive, packed into loaves, wrapped in wax paper, and stored frozen in a dry-ice chest for one to two days. They were chiseled open, and the internal color was inspected immediately.

Methylene blue reduction. Conditions were made anaerobic by gelling the reaction components in agar *(13)*. Each tube contained the following: 2.5 ml. 1.6% agar in 0.067 M potassium phosphate buffer, pH 7.0; 0.5 ml. 1 M substrate; 0.5 ml. 1:4000 methylene blue; and 1.0 ml. 20% (w/v) homogenate of muscle, prepared by the Potter-Elvehjem method *(13)*. The meat sample was cow rib muscle frozen within 24 hours of slaughter and thawed 1 hour before use. The assay temperature was 40°C.

Measurement of oxygen consumption. Several key Krebs cycle enzymes were assayed manometrically at 37°C. in the conventional Warburg apparatus. All substrates were present at a final concentration of 0.07 M. The acids were added as neutralized sodium salts and histidine as the dihydrochloride. Magnesium sulfate was present in the pyruvate system at 0.003 M. Nicotinamide and monosodium glutamate were present in the fumarate and lactate systems at 0.07 M. Adenosine triphosphate was added to the citrate, glutamate, pyruvate, and acetate systems at a final concentration of 0.004 M. Cytochrome c was added to every system at 6×10^{-5} M. Diphosphopyridine nucleotide was provided at 2.10^{-3} M, triphosphopyridine nucleotide at 1.33×10^{-3} M and Coenzyme A at 236 Lipmann units per flask to every system except the succinate. Every flask contained 1.6 ml. of a 10% homogenate of cow short loin muscle *(semitendinosus)* in 0.28 M Sorenson phosphate buffer, pH 7.4. The final phosphate concentration was 0.135 M and the final tissue concentration was 160 mg. wet weight per flask. Readings were taken every 10 minutes for 40 minutes, and the enzyme activities were expressed as Q_{O_2} (mm.^{3} O_2 consumed per hour per mg. dry weight of enzyme).

RESULTS

For the inhibitor study, an important consideration is the choice of a sufficiently large amount of inhibitor to work under the conditions of low temperature and lack of mobility. According to Bate-Smith *(3)*, beef contains about 0.005% cytochrome c. This is about 0.4 micromoles per 100 g. If the cytochrome c content is any index to the content of the enzymes, then the additive concentrations used here would provide more than 2000 molecules of inhibitor to each molecule of enzyme.

Tested at a level of 176 mg. per 100 g. tissue were arsenite, fluoride, fluoracetate, malonate, p-amino benzoate, mercuric chloride, aniline, 2,4-dinitrophenol, hydroquinone, thiourea, and glucose. Sodium acetate was studied at 107 and tetrasodium pyrophosphate at 265 mg. per 100 g. tissue.

Only malonic acid, among the enzyme inhibitors, provided a bright red interior color. Since this is a highly specific competitive inhibitor of succinic dehydrogenase, the enzyme is regarded as active in beef.

In the presence of several additives there was found a substantially greater brown region than was possessed by the controls. The enzymes blocked by these additives are regarded as showing very limited activity, if any. Three glycolytic enzymes in this category are phosphoglucomutase, phosphatase, and enolase. The oxidative reactions in this category are lactic dehydrogenase, malic dehydrogenase, and the conversions of pyruvate to S-acetyl coenzyme A, S-acetoacetyl coenzyme A to S-acetyl coenzyme A, citrate to oxalosuccinate, citrate to malate, α-ketoglutarate to succinyl coenzyme A, and succinyl coenzyme A to succinate. Tyrosinase is in this category.

Where a chemical produced no effect different from the control, the presumption is that the corresponding enzyme is inactive. In this category are L-glutamic acid oxidase, catalase, phosphorylase, glucose, fructose, and formate dehydrogenases, fatty acid autoxidation, and the coupling of oxidation to phosphorylation.

The substrates for the methylene blue reduction study were glucose and the sodium salts of acetic, glutamic, formic, α-glycerophosphoric, succinic, and tartaric acids. In 17 hours the addition of only 2 substrates provided the necessary sparking for complete reduction. Succinic dehydrogenase and α-glycerophosphate dehydrogenase were both active in steer muscle, but only succinic dehydrogenase displayed activity in cow

TABLE 1

Muscle dehydrogenations assayed by oxygen consumption

Substrate	Q_{O_2}	Relative rate
Succinate	13.5	100
Glycerophosphate	7.0	52
Citrate	2.5	18
Glutamate	1.9	14
Lactate	1.7	13
Fumarate	0.6	4
Acetate	0.6	4
Pyruvate	0.6	4
Histidine	0.0	0

muscle. A blanket negative statement cannot be made regarding the other enzymes, because, unlike the above two, these all require the participation of one or more coenzymes. A positive display of activity would have verified the presence of these coenzymes.

Table 1 gives the results of the manometric studies. Succinic dehydrogenase and α-glycerophosphate dehydrogenase display markedly stronger activity under optimum conditions than other respiratory enzymes.

Succinic dehydrogenase and α-glycerophosphate dehydrobenase are both linked to the cytochrome oxidase system. In the methylene blue study, the dye replaced not only oxygen, but also the entire cytochrome carrier apparatus between succinate and oxygen. In the inhibitor and manometric experiments, however, a positive result rested upon the functioning of cytochrome oxidase. To buttress this, beef muscle cytochrome oxidase was separately assayed manometrically *(10)* and spectrophotometrically *(1)*. Appreciable activity was found in homogenates and minces, at pH 7.4 and pH 5.5 and at both 25°C. and 37°C.

DISCUSSION

There is a practical distinction between the inactivity of an enzyme as a result of its deterioration and inactivity as result of substrate dearth. The first is irreversible. The second is reversible when and if new molecules of substrate are created by coupled metabolic reactions or when the substrate is deliberately introduced from the outside. Failure to elicit activity in the presence of added substrate and cofactors, as in several instances of the present study, can be attributed to enzyme destruction or absence of the enzyme in the functioning muscle.

The study of Andrews, Guthneck, McBride, and Schweigert *(2)* and the present manometric and methylene blue experiments underscore the stability of the beef muscle succinoxidase enzymes. The uncertain supply of succinate thus becomes a limiting factor. That a supply exists at all is supported by the malonate inhibitor results, which were obtained in the absence of added substrate. Szent-Gyorgyi *(11)* estimated that a few hundredths of a milligram of succinate would stabilize the entire respiration. He reported the muscle succinate level as 10 mg. per 100 g., although Frohman, Orten and Smith *(5)* found less. The original supply of substrate in an excised muscle would be ephemeral under favored physiological conditions of temperature and pH, but the low temperature and less favorable pH of meat undoubtedly curb the depletion.

The effects of a surviving succinoxidase system may extend to the physical state of fresh meat and the color of cured meat. Szent-Gyorgi *(12)* discovered a complete parallelism between the oxidation of succinate and

the contractile state of actomyosin fibers. Maximal contraction was accompanied by maximal enzyme activity, partial contraction by correspondingly less activity, and absence of contraction by absence of activity. The character of magnesium and calcium ion inhibition was the same for both processes.

Sato and Egami *(9)* identified the nitrate reductase system of *E. coli* with cytrochrome b, which is capable of coupling with succinic dehydrogenase. This type of reaction might conceivably occur during curing.

SUMMARY

A survey was made of the surviving oxidative enzymes of meat frozen within 18 hours of slaughter and thawed before assay. Three methods of identification were used: the action of specific inhibitors on enzyme-promoted color changes, the capacity to reduce methylene blue, and the rate of oxygen consumption. Succinic dehydrogenase, α-glycerophosphate dehydrogenase, and cytochrome oxidase were the only appreciably active enzymes. The significance of these results is discussed.

LITERATURE CITED

1. Albaum, H. G., Tepperman, J., and Bodansky, O. A spectrophotometric study of the competition of methemoglobin and cytochrome oxidase for cyanide in vitro. *J. Biol. Chem.*, 163, 641 (1946).
2. Andrews, M. M., Guthneck, B. T., McBride, B. H., and Schweigert, B. S. Stability of certain respiratory and glycolytic enzyme systems in animal tissues. *J. Biol. Chem.*, 194, 715 (1952).
3. Bate-Smith, E. C. The chemical composition of mammalian and avian meat. *Chem. Ind.*, 61, 373 (1942).
4. Brooks, J. Color of meat. *Food Research*, 3, 75 (1938).
5. Frohman, C. E., Orten, J. M., and Smith, A. H. Chromatographic determination of the acids of the citric acid cycle in tissues. *J. Biol. Chem.*, 193, 277 (1951).
6. Lavers, C. G. Discoloration of packaged red meat. *Modern Packaging*, 21, (1948).
7. Lawrie, R. A. The activity of the cytochrome system in muscle and its relation to myoglobin. *Biochem. J.*, 55, 298 (1953).
8. Lemberg, R., and Legge, J. W. *Hematin Compounds and Bile Pigments.* 1949, Interscience Publishers, New York, N. Y.
9. Sato, R., and Egami, F. Nitrate reductase. *Bull. Chem. Soc. Japan*, 22, 137 (1949).
10. Schneider, W. C., and Potter, V. R. The assay of animal tissues for respiratory enzymes. II. Succinic dehydrogenase and cytochrome oxidase. *J. Biol. Chem.*, 149, 217 (1943).
11. Szent-Gyorgyi, A. *On Oxidation, Fermentation, Vitamins, Health, and Disease.* 1939, The Williams and Wilkins Co., Baltimore, Md.
12. Szent-Gyorgyi, A., *Muscle Contraction.* 1947, Academic Press, Inc., New York, N. Y.
13. Umbreit, W. W., Burris, R. H., and Stouffer, J. F. *Manometric Techniques and Tissue Metabolism.* 2nd Ed., 1949, Burgess Publishing Co., Minneapolis, Minn.
14. Urbain, W. Meat and Meat Products. *Food.* Vol. II, ed. by Jacobs, M., 1951, Interscience Publishers, New York, N. Y.

THE COMPARATIVE EFFECT OF IONIZING RADIATIONS AND HEAT UPON THE STARCH CONTAINING CELLS OF THE POTATO TUBER[a]

EDITH A. ROBERTS AND BERNARD E. PROCTOR

Department of Food Technology, Massachusetts Institute of Technology, Cambridge

(Manuscript received December 28, 1954)

Ionizing radiations have assumed great importance in the investigation of fundamental problems of a biological, biophysical, or biochemical nature. This importance is evident if one examines recent literature in which ionizing radiations have elucidated structures at the cellular and intracellular level as well as biological processes and mechanisms.

The fact that high temperature would produce chemical changes has long been known, and Proctor and Goldblith *(18)* have indicated that ionizing radiation of suitable intensity would produce chemical changes in plant and animal materials subjected to high-voltage x-rays and cathode rays.

Direct observations of the changes in cellular and intracellular structures effected by ionizing radiations have been made, using optical means such as the light or electron microscopes, and correlating this with previous knowledge of structure to determine structural features otherwise difficult to obtain. The investigation described herein utilizes the light microscope in a detailed study of the comparative effect of temperature and of 3 m.e.v. cathode rays upon the structure of the walls of the starch-containing cells of the potato.

It has been shown that high-energy cathode rays effect chemical and physical changes without a marked change in temperature. There is a rise of measurable temperature of only 2°C. for each 1×10^6 rep. Studies of the effect of temperature and high-energy cathode rays upon the starch-containing cells of the potato tuber were initiated with the hope of furthering knowledge concerning both radiation changes and starch cell structure.

As far as can be determined, there has been no published literature that indicates the comparative effect of temperature and high-energy cathode rays on the walls of the predominately parenchymatous cells of the potato tuber. Before such treatment may be evaluated, however, the physical and chemical nature of the walls under normal conditions should be considered. In no article has that fact been presented more clearly than in the one on "Histological Changes Induced in Fruits and Vegetables by Processing" by Weier and Stocking *(25)*.

The investigations of Artschwvager *(1)*, Barrows *(2)*, Hermans *(12)*, Kertesz *(13)*, Branfoot-Carre *(3)*, and Frey-Wyssling *(9)* indicate that in cell walls there is a middle lamella, which is composed primarily of calcium pectate; next to this is the primary wall, consisting of a cellulose framework which is interspersed with pectins and hemi-cellulose substances and possibly proteins. The secondary wall is mostly cellulose and is made

[a] Contribution No. 245 from the Dept. Food Tech., Mass. Inst. Tech., Cambridge, Mass.

up of (a) an outer layer; (b) a central layer made up of many lamellae; these lamellae are of some 3-4000 A in diameter; and (b) an inner layer. These 3-4000 A lamellae are composed of fibers which are again composed of microfibrils of some 250-300 A in diameter and the microfibrils in turn of micellae strands of 60 A in diameter, with interfibrillar capillaries of 100 A. The micellar strands, according to Mühlethaler *(14) (15)* appear to be real morphological units.

Thimann *(23)* postulated that some auxin causes a change in the metabolism and therefore the cell wall depends on metabolic alterations within the cell, which must, as an indirect effect, produce an enzyme which stimulates the formation of new microfibrillar cellulose strands. This auxin acts as a feeble acid in swelling pectins and it is the cytoplasm that creates the lamellae in the building of the cell wall.

It is known that oxidants, such as hydrogen peroxide, destroy not only the middle lamella, and the primary wall, but may attack the outer layer of the secondary wall, where an "amorphous substance" is present interwoven with the fiber, possibly within the fibrils. Bonner *(4)* found that a well marked cell envelope remains after treating with pectinase and cellulase enzymes, which seemed like protein and probably was a complete envelope, which is in agreement with the findings of Thimann and Bonner *(23)*. Wood, Gold, and Rawlins *(26)*, by the use of pectic enzymes, removed the pectin constituents from cell walls. One square centimeter pieces of parenchyma tissue, including that of potato, were used in their study. They confirmed the prevalent idea that much of the pectic material within a primary wall is located between the cellulose fibrils. Ranby *(19)* found the micelles to be bundles of 100-500 cellulose molecules of a width of about 70 A. Preston *(16) (17)* recognizes that the question of the relation of the micelles and microfibrils is still under discussion. The additional postulation of Mühlenthaler *(14) (15)* that there is an intimate connection between protoplasm and the developing layers of plant cell walls gives some idea of the possible variation in the effect of temperature and high-energy cathode rays upon the walls of the parenchyma cells in general and particularly upon the pectinaceous substances present. The presence of a pectinaceous substance not only in the middle lamellae, but throughout the fibrils of the walls, makes any treatment which may change or destroy this substance have a profound effect upon the quality of the potato "granules" produced.

MATERIALS AND METHODS

Tubers of the potato (*Solanum tuberosum* L. var. Katahdin) were used in most of the determinations, and when other varieties were used the results were comparable. The potatoes were kept at 8.2°C. in accordance with the recommendation of Denny and Thornton *(6)*, in order that the starch content be held as constant as possible. In all experiments, segments of the tubers $10 \times 10 \times 10$ mm.. were cut from the parenchymatous areas. Ten each were packed in polyethylene tubes, which were heat-sealed, except in the experiments in which segments were placed in direct contact with a water medium.

A. Temperature series. The temperature series were conducted in the following ways and the pectin and cellulose content examined.

1. *Water medium*

The potato segments were placed directly in water. The water heated to 100°C.

at 10° rise per minute. Segments were removed at each 5° rise in temperature and the tests applied. This treatment is referred to as "cumulative."

2. *Air medium*

a. Potato segments were sealed in polyethylene packets, the packets placed in water, heated, and tested as in the water medium.

b. Potato segments were packed as in 2a, but dipped at each of the 5° intervals of temperature for 2 seconds, this being the time corresponding to the time of the irradiation doses. These are called "temperature equivalents." The packets were immediately placed in ice water as were the packets in the irradiation series.

B. Irradiation series.[b] In this series the segments were irradiated in an ice-water bath with a total dose of 3.0×10^6 rep (rep is taken equivalent to 83 ergs per gram). This dose was given in twelve equal portions of 0.25×10^6 rep at intervals of 2 seconds.

Microchemical methods for the determination of the wall substances. Microchemical methods were those of Eckerson *(6)*, unpublished, Rawlins *(20)*, Gomori *(11)*, and Farr *(8)*, personal communication.

1. *Cellulose*

a. H_2SO_4–I_2KI method.

A drop of I_2KI solution was placed on the mount and allowed to dry at the edge of the coverglass. A small drop of 72% H_2SO_4 was added at edge of cover slip and drawn over the mount. The reaction may be checked if desired by the addition of a drop of glycerin. (Solution of I_2KI is 0.3 g I_2, 1.3 g KI and 100 cc. water. This was diluted further by 3 parts of solution to 1 part water.) (Cellulose becomes bright blue in color and a purplish blue, if cellulose is associated with pectinaceous substances.)

b. Chlor-zinc iodide (color is the same as [a]).

Fifty grams zinc chloride, 16 grams iodide, 17 cc. water. Excess of iodine added and allowed to stand several days. The supernatant liquid was used.

2. *Pectic substances*

(Pectinaceous substances become wine red in presence of ruthenium red.)

a. Color reaction.

Ruthenium red 1 ÷ 5000 was used. This may vary to 1 ÷ 10,000, as suggested by Preston *(16)*.

b. General method.

Heat in 2% HCl for 1 hour. The pectic substances are changed to pectins (soluble) and pectic acid (insoluble) is set free. Calcium pectate is broken down and $CaCl_2$ formed. Wash and only pectic acid remains. Treat with 2% ammonia and pectic acid dissolves after about 1 hour. (Soluble in 0.5% ammonium oxalate after treatment with acid alcohol—50% alcohol and 50% HCl—for 12 hours.) Preston *(17)*.

When stained again with ruthenium red, there should be no pectic substance remaining, but if so, a longer time was taken at each step.

While the detection of pectic substances is questionable, according to Kertesz *(13)*, "ruthenium red is still the best stain recommended for the observation of pectic constituents of plants under the microscope." The confirmation can also be made by treating with 5% HCl and 5% KOH to hydrolyze proto-pectin; then treat with acid ethanol and dilute NH_4OH to dissolve the acid in addition to using 0.05% ammonium oxalate to remove all pectic substances.

Photomicrographic equipment. 1. Leitz inclined binocular microscope BS 47/92K equipped with interchangeable monocular photographic tube with lateral binocular observation tube.

2. Micro-ibso attachment equipped with compensating 10 × periplanatic eyepiece, a main housing with built-in shutter, and a lateral viewing telescope. Above the shutter is a conical extension tube with an achromatic lens system.

---

[b] Through the courtesy of Dr. John G. Trump the packages were exposed to various doses of high-energy cathode rays in the Van de Graaff generator, operating at 3,000 kev., as described by Goldblith and Proctor *(10)*, and Trump, Wright and Clarke *(24)*.

3. Leitz apochromatic objective 40× (4 mm.) N.A. 0.95.
4. Leitz Wasser immersion objective 90× (1 mm.) N.A. 1.20.
5. Leitz aplanatic achromatic condenser in centering mount N.A. 1.40.
6. Leitz graduated mechanical stage.
7. An Edgerton *(7)* (unpublished) high-speed (one-thousandth of a second) electronic flash-lighting microscope lamp.

RESULTS

A. Temperature series. 1. Water medium.

Table 1 indicates the effect of "cumulative" temperature, in the water medium, upon the cellulose and pectin content of the middle lamellae and walls. The primary wall appeared to remain intact and is of a protein nature, in agreement with the tests of Thimann and Bonner *(23)*.

TABLE 1

Effect of cumulative temperature on walls of potato tuber parenchyma cells.
A. Temperature Series. Water Medium.

Max. temp. reached °C.	Middle lamella		Primary and secondary wall		
	Structure	Content pectin	Structure	Content	
				Pectin	Cellulose
0	Compact	Much	Compact	Much	Purplish blue
30	Compact	Much	Compact	Much	Purplish blue
40	Compact	Much	Less compact	Much	Purplish blue
50	Breaking	Little	Less compact	Much	Purplish blue
60	Broken	None	Laminated	Less	Less purple
70	Absent	None	Thin layers	Less	Less purple
80	Absent	None	Thin layers	Less	Good blue
90	Absent	None	Thin layers	Particulate	Deep blue
100	Absent	None	Thin layers	Very little	Blue particles

At 55°C. the pectinaceous substance of the middle lamellae is altered. Much of the pectic substances in the walls has been removed as the walls, when tested, are of the blue color of cellulose with no associated pectic substance. When heated to 60°C. the starch is disintegrated. There is much variance in the walls and the cells may be lightly held together by plasmodesmata strands, which may account for Mühlenthaler's *(14)* suggestion of the "intimate connection" of protoplasm with walls.

When the segments are heated in water to 100°C. the middle lamella is entirely altered, and there is little, if any, pectic substance remaining in the walls, and the starch grains are disintegrated.

The effect of a water medium at a temperature around 60°C., plus or minus 5°C., is the complete alteration of the pectic substance of the middle lamellae, and at 100°C. the pectic substance in the walls appears to have been entirely deleted, for the test for cellulose, after this treatment, is that of a clear blue—typical of pure cellulose—not the green or purplish blue seen when cellulose is associated with pectins.

Since there is a wide range in the age and content of the cells, and hence considerable divergence in their wall development, there is a variation in the illustrations that can be obtained. Table 1 represents the average of some 100 observations.

2. Air medium.

a. The time the packeted segments were in water was the same as the segments in direct contact with water, as in A-1.

In Table 2 the effect of temperature in an air medium is indicated, the amount of moisture present being only that which is present in the fresh potato cells. The temperature showed little effect upon either the middle lamellae or the walls until it reached nearly 90°C. It reached 95°C. before the middle lamellae were altered.

B. Irradiation series. In this series the segments sealed in polyethylene packets were exposed to doses of 0.25×10^6 rep for 2 seconds, over ice-water, and cooled between

TABLE 2

Effect of cumulative temperature on walls of potato tuber parenchyma cells. A. Temperature Series. Air Medium and Cooled Immediately after 5° Exposure Intervals.

Max. temp. reached °C.	Middle lamella		Primary and secondary wall		
	Structure	Content pectin	Structure	Content	
				Pectin	Cellulose
0	Compact	Much	Compact	Much	Purplish blue
60	Compact	Much	Compact	Much	Purplish blue
70	Breaking	Little	Compact	Much	Purplish blue
80	Breaking	Little	Compact	Much	Purplish blue
90	Breaking	Little	Laminated	Much	Purplish blue
100	Absent	None	Thin layers	Very little	Blue particles

doses until a total of 3×10^6 rep was given. Assuming that the temperature was raised 2°C. for each million roentgens of radiation applied, the amount of heat gradually increased but the intensity was far below that which would have any observable effect upon either the middle lamella or the walls, as shown in Table 2.

Table 3 indicates the effect of irradiation upon the walls at the 0.25×10^6 rep increments. While there is much variation in the age and development of the walls in even the 10 mm. segments of a potato, in general, after a dose of 1×10^6 rep, the walls may become laminated and the amount of pectic substance in the middle lamellae shows a decrease. However, there is sufficient amount present to hold the cells together, while the starch grains remain unchanged in form, as shown in Figure 1. The cells are seen to be separating after a dose of 2.0×10^6 rep, as shown in Figure 2. The form of the starch grain, however, is unchanged, and it remains unchanged even after a dose of 10.0×10^6 rep.

While the middle lamellae apparently are altered, the cells are held together by the plasmodesmata, but around 2.5×10^6 rep and often above a dose of 2.0×10^6 rep these protoplasmic connections are broken.

TABLE 3

Effect of high-energy cathode rays on walls of potato tuber parenchyma cells. B. Irradiation Series. 1. In doses of $\frac{1}{4} \times 10^6$ rep intervals up to 3×10^6 rep. (Rep or Roentgen-equivalents-physical.)

Dose $\times 10^6$ rep.	Middle lamella		Primary and secondary wall		
	Structure	Content pectin	Structure	Content	
				Pectin	Cellulose
Control	Compact	Much	Firm	Much	Purplish blue
0.25	Compact	Much	Firm	Much	Purplish blue
0.50	Compact	Much	Laminated	Fair amount	Purplish blue
0.75	Less compact	Much	Laminated	Fair amount	Purplish blue
1.00	Laminated	Fair amount	Laminated	Fair amount	Purplish blue
1.25	Laminated	Fair amount	Laminated	Fair amount	Faint blue
1.50	Laminated	Fair amount	Laminated	Some	Faint blue
1.75	Cells lightly held together	Little	Laminated	Some	Faint blue
2.00	Most cells apart	Little	Laminated	Very little	Faint blue
2.25	Cells apart	Little	Laminated	Very little	Blue
2.50	None	Little	Grainy	Very little	Blue
2.75	None	Very little	Grainy	Very little	Blue
3.00	Absent	None	Grainy	In particles	Blue

Figure 1. Photomicrograph of cells which have received a dose of 1×10^6 rep. The walls are quite firm and the starch grains have retained their form.

DISCUSSION

The chemical nature and physical attributes of the walls of living plant cells are largely known, but there has been no published material that we have found that gives the comparative effect of temperature and high-energy cathode rays on the walls of the cells of the potato tuber, with their predominately parenchymatous cells or upon the starch grains within them.

In this study the cell walls of the starch-containing cells were found to contain pectinaceous, proteinaceous, and cellulose substances; the middle lamellae composed of calcium pectate, the primary wall possibly containing some protein, and the secondary walls composed mainly of cellulose and pectinaceous substances.

In comparing the effect of temperature and high-energy cathode rays on the walls of the parenchymatous cells of the potato tubers, it was found that if the segments were heated in a water medium, the middle lamellae are altered at a temperature around 60°C. There is a wide range, some

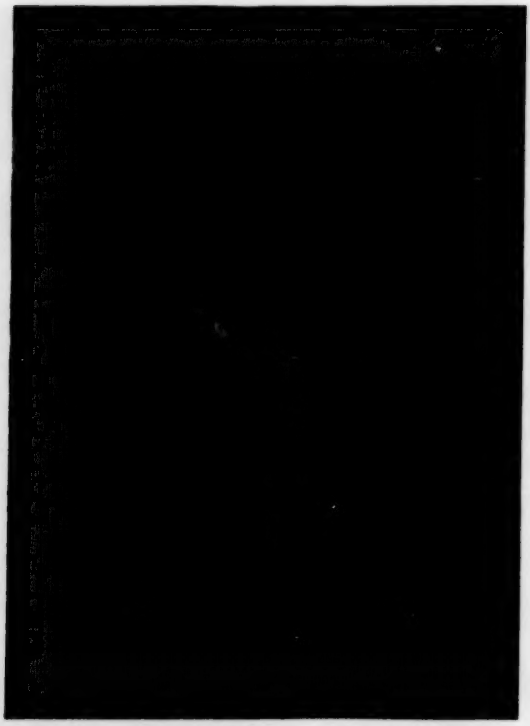


Figure 2. Photomicrograph of cells which have received a dose of 2.0×10^6 rep. The middle lamellae have been altered but the grains retain their form.

$\pm$ 10°, in the effectiveness of temperature. This is to be expected, for there is a wide range in the age of the cells in any tissue and hence a variation in the chemical and morphological development of the cells, which would result in varying effects.

The alteration of the calcium pectate of the middle layer causes the tissue of the potato segments to separate into isolated cells. This separation is not so obvious unless pressure is applied to the 10 mm. cubic segments. The slightest pressure, however, after the calcium pectate is altered causes the cells to separate; but until that pressure is applied, the cubical segments retain their shape. This retention of form is due to the plasmodesmata, which exist as a network, from the cytoplasm of one cell through the walls to the adjoining cells. The slightest pressure, when the walls are weakened, breaks these delicate protoplasmic threads.

At the same time that the middle lamellae are being altered by temperature, the pectins in the primary and secondary walls are changed in such a way that the compact lamellae are separated into the microfibrils, and there is a decrease in the amount of pectin about the cellulose particles in the microfibrils which results in the walls being easily fractured.

As the pectins are altered, the presence of the cellulose in the walls becomes more apparent. This is very evident, as the walls of the segments became brighter blue, rather than purplish blue, when they were tested for cellulose and pectins at each 5°C. increment in temperature. The cellulose is so intermingled with the pectic substances in the walls that when the pectic substance is being removed or modified, the walls are weakened and become fragmented or lamellate. The starch grains, at the same time, lose their structure and become a disintegrated gummy mass which is spread over the fragmented walls.

At 90° C., in a water medium, the cellulose is reduced to sheets of small particles of less than one micron in diameter, and the pectin, if present, is so small in amount that it cannot be detected by the light microscope. When the segments were heated in air-filled sealed packets of polyethylene comparable to those used in the irradiation series, and the packets placed in water, the temperature could be raised to 90°C. before the pectins of the middle lamellae were altered, and could be raised to 100°C. before the starch grains were disintegrated into 1 micron units. The temperature within the sealed packets was found to be the same as that of the surrounding water, the difference being that in the packets the cells had only the sap of cut cells as a liquid medium.

Irradiation doses from 2.0×10^6 rep to 3.0×10^6 rep altered the calcium pectate middle lamellae and possibly the pectinaceous content of the walls at a temperature not exceeding 22°C. This amount of irradiation appeared to have little, if any, effect upon the cellulose or the starch grains.

It appears that the pectins are destroyed at a much lower irradiation dose than cellulose; for Saeman, Millet, and Lawton *(21)* found that cellulose was depolymerized at about 5.0×10^8 rep.

The irradiation series shows that it is the effect of irradiation and not that of temperature that alters the pectic substances in the middle lamellae and walls; for at a dose of 2.5×10^6 rep (equivalent temperature rise of 5°C. only), the actual temperature to which segments were subjected being 20°C., the clear blue of the cellulose is evident and the cells are easily separated. The starch grain, however, retains its structure at this level of irradiation.

While the pectic substance in the middle lamellae is altered from 1-2.0×10^6 rep, the plasmodesmata are not destroyed until a dose of 2.5×10^6 rep is received, and the starch grain retains its form even beyond a dose of 10.0×10^6 rep.

SUMMARY

In a water medium, the middle lamellae of the parenchymatous starch-containing cells of the potato tuber are altered as soon as the temperature reaches 55-65°C. The pectic substances in the walls are altered at 70°C. The cellulose content of the walls is not destroyed at this temperature, but is so weakened that the walls are fractured and no longer hold within them the substance of the starch grains. The grains, which also have been affected by the temperature, lose their form and are released as a sticky mass.

In an air medium, or in waterproof polyethylene packages, the middle lamellae are not altered until 90°C. while the walls are not weakened until

the temperature reads 100°C. The effect upon the starch grains is similar to that in the water medium, but at a higher temperature.

An irradiation dose of cathode rays of 2-3.0 × 10^6 rep, with a resulting temperature of less than 22°C., altered the middle lamellae, but the walls are not weakened to the breaking point and the form of the starch grains is retained.

The finding that a dose of from 1-2.0 × 10^6 rep will effect the separation of the cells without weakening the walls to the breaking point, and without changing the physical appearance of the starch, makes the use of high-energy cathode-ray technique resulting in physical and chemical changes in plant tissues worth investigation.

Further histochemical studies on both potato and other plant tissues are in progress.

LITERATURE CITED

1. Artschwvager, E. Studies on the potato tuber. *J. Agr. Res.*, 27, 809-836 (1923).
2. Barrows, F. L. Cellular membranes from various parts of the plant kingdom. *Cont. B.T.I.* 11, 61-82 (1939).
3. Branfoot, M. H. (M. H. Carré) *Food Investigation-Special Report* 33. Dept. Sci. and Ind. Res. (1929) London.
4. Bonner, J. *Plant Biochemistry.* 1950, Academic Press, Inc. New York, N. Y.
5. Denny, F. E., and Thornton, N. C. Potato varieties; sugar-forming characteristics of tubers in cold storage, and suitability for production of potato chips. *Cont. B.T.I.* 12, 217-252 (1941).
6. Eckerson, S. H. *Outlines of Microchemistry.* (Unpublished) (1940).
7. Edgerton, H. W. High-speed electronic flashlighting equipment. (Unpublished).
8. Farr, W. E. (Communication by letter, 1952).
9. Frey-Wyssling, A. (Ed.) *Deformation and flow in biological systems.* 1952, North-Holland Publishing Co., Amsterdam.
10. Goldblith, S. A., and Proctor, B. E. Effect of high-voltage x-rays and cathode rays on vitamins (riboflavin and carotene). *Nucleonics*, 5 (2), 50-58 (1949).
11. Gomori, G. *Microscopic Histochemistry.* 1952, The University of Chicago Press, Chicago, Illinois.
12. Hermans, P. H. *Physics and Chemistry of Cellulose Fibers.* 1949, Elsevier Publishing Co., New York, N. Y.
13. Kertesz, Z. I. *The Pectic Substances.* 1951, Interscience Publishers, New York, N. Y.
14. Mühlenthaler, K. Electron Micrographs of Plant Fibers. *Biochim. et Biophys. Acta*, 3, 15-25 (1949).
15. Mühlenthaler, K. Electron microscopy of developing plant cell walls. *Biochim. et Biophys. Acta*, 5, 1-9 (1950).
16. Preston, R. D. *The Molecular Architecture of Plant Cell Walls.* 1952, J. Wiley & Sons, New York, N. Y.
17. Preston, R. D. Biophysical aspects of polysaccharide structure in plants. *Progr. in Biophys.*, 2, 17-50 (1951).
18. Proctor, B. E., and Goldblith, S. A. The effect of super-voltage cathode rays on the nonenzymatic browning reaction of dried fruits and on chemical compounds pertaining thereto. *Science*, 109, 519-522 (1949).
19. Ranby, B. G. Fibrous macromolecular systems. Cellulose and muscle. The colloidal properties of cellulose micelles. *Faraday Society Discussion*, 11, 158-161 (1951).
20. Rawlins, T. E. *Techniques of plant histochemistry and virology.* 1952, National Press, Milbrae, California.
21. Saeman, J. F., Millet, M. A., and Lawton, E. J. Effect of high-energy cathode rays on cellulose. *Ind. Eng. Chem.*, 44, 2848-2852 (1952).
22. Thimann, K. V. Plant Hormones, growth and respiration. *Bio. Bull.*, 96, 296-300 (1949).

23. THIMANN, K. V., AND BONNER, J. The mechanism of the action of the growth substance of plants. *Proc. Roy. Soc. B.* London, 113, 126-149 (1933).
24. TRUMP, J. G., WRIGHT, K. A., AND CLARKE, A. M. Distribution and ionization in materials irradiated by two and three million-volt cathode rays. *J. App. Phys.*, 21, 345-348 (1950).
25. WEIER, T. E., AND STOCKING, C. R. Histological Changes Induced in Fruits and Vegetables by Processing. *Advances in Food Research*, 2: 297-342 (1949).
26. WOOD, R. K. S., GOLD, A. H., AND RAWLINS, T. E. Electron microscopy of primary cell walls treated with pectic enzymes. *Am. J. Bot.*, 39, 132-133 (1952).

DENATURATION OF PROTEINS OF EGG WHITE AND OF FISH AND ITS RELATION TO THE LIBERATION OF SULFHYDRYL GROUPS ON FROZEN STORAGE

SAEED AHMAD HUSAINI AND FRITHIOF ALM
Swedish Institute for Food Preservation Research, Gothenburg, Sweden

(Manuscript received January 20, 1955)

During the last few years there has been a rapid expansion in the freezing method of fish preservation, but this method has brought with it new problems. Storage of fish at temperatures below freezing retards bacterial metabolism and enzymatic reactions considerably, but storage at these temperatures is not the complete solution to the problem of maintaining quality as near as possible to the initial fresh product. Deterioration does occur during frozen storage. One of the main causes is said to be protein denaturation. Several factors affect the progress of denaturation, chiefly, temperature, freshness of fish before freezing, and rate of freezing.

It has been observed that in certain proteins in the native state the total number of sulfhydryl groups is not available to chemical reagents employed for their determination. It is only after denaturation that these proteins make all their sulfhydryl groups available to chemical reagents. Not all proteins, however, show this behaviour. Moreover, the amount of sulfhydryl groups liberated on denaturation depends on the nature of protein, denaturing agent employed, temperature, pH and the like. In other words there are several degrees of availability of sulfhydryl groups.

Since the denaturation of proteins and liberation of chemically reactive sulfhydryl groups are processes closely linked to each other, the degree of availability of sulfhydryl groups can be used as a measure of the extent of denaturation of these proteins, provided that all other factors that affect the liberation of these groups are kept constant. Frozen fish when stored for a certain length of time undergoes deterioration which is said to be due partly to the denaturation of proteins. Hence with progressive denaturation of proteins of frozen fish more and more sulfhydryl groups should be liberated, or in other words, the amount of "masked" sulfhydryl groups should decrease. This is the chief point of view that we have taken into consideration in our study of protein denaturation. The difference between the total content of sulfhydryl groups of proteins as determined after the addition of a denaturing agent—such as urea, Duponol PC, guanidine hydrochloride, etc.—and the amount of free available sulfhydryl groups gives the value of "masked" sulfhydryl groups. The term "masked" sulfhydryl groups is used throughout this paper to indicate this value. The criterion of decrease in solubility of proteins has also been employed as an index of protein denaturation. We have used white of hen's eggs and fresh cod fillets for our study.

EXPERIMENTAL

Experiments with white of egg: White of fresh or 2-3 days' old hen's eggs was collected and mixed together gently until the membranes were broken and the mass of

egg white became homogeneous. Care was taken to avoid too much shaking and foaming so as not to cause denaturation. Samples of about 30 g. each were placed in 50-ml. Erlenmeyer flasks which were stoppered tightly to prevent loss of moisture. The samples were then divided into 5 lots and stored at 0°C. (32°F.), and frozen at –5°C. (23°F.), –10°C. (14°F.), –15°C. (5°F.) and –20°C. (–4°F.) and stored at the same respective temperatures. Samples were taken out from each lot from time to time for analysis. The exact procedure was as follows:

The samples were allowed to thaw at room temperature and the entire contents of the Erlenmeyer flask were weighed accurately into a 100-ml. volumetric flask, dissolved gently in distilled water and finally made up to volume. Too much shaking and agitation were avoided. Twenty ml. of this egg white solution were used each time for the determination of sulfhydryl groups before and after denaturation. Duplicate determinations were made in each case.

Sulfhydryl groups were determined by the amperometric titration method of Kolthoff and Harris *(10)* as adapted to proteins by Benesch and Benesch *(2)*. This method was tested in our laboratory and found to be quite satisfactory. Known amounts of pure cysteine hydrochloride alone when titrated by this method were recovered to the extent of 99.5%. But when the known amounts of pure cysteine hydrochloride were added to the known samples of egg white and the mixture titrated, the sensitivity of the method was reduced, the recovery of cysteine hydrochloride being 90-92%. This, however, was quite satisfactory for our work. The apparatus and procedure used were essentially the same as those described by Kolthoff and Harris *(10)*. However, since we assayed quantities considerably smaller than those estimated by these authors, microburettes and a more sensitive galvanometer were employed.

To 20 ml. of the above egg white solution 20 ml. of 0.1 M phosphate buffer (pH 7) were added followed by 150 ml. of an aqueous solution of 0.05 M ammonium nitrate and 0.25 M ammonia. The egg white was then titrated in an atmosphere of nitrogen with 0.005 N silver nitrate solution. The end point was obtained by plotting current readings against volume of silver nitrate added and noting the point of intersection of the two straight lines *(2, 10)*.

For the denaturation of egg white proteins Duponol PC was used. To 20 ml. of the egg white solution were added 20 ml. of 0.1 M phosphate buffer (pH 7) plus 1 g. of Duponol PC which was dissolved separately in a small amount of water and then added to the egg white. The mixture was allowed to stand for half an hour under nitrogen and then titrated with silver nitrate as described.

The aqueous egg white solution was centrifuged at room temperature for 25 minutes at 1000 r.p.m. Two ml. of the supernatant liquid were taken for the determination of soluble nitrogen according to the method of Kjeldahl-Lundin-Ellburg *(11)*.

Experiments with cod: Fillets from small fresh cods were used in this experiment. Cods were filleted as soon as they were landed, cooled in ice, and brought to the laboratory as soon as possible. Each of these fillets was divided into 4 pieces of 25-30 g. each, each piece going into a different lot. In this manner 4 homogeneous lots were obtained. Pieces of cod fillets from each lot were then placed in small cellophane bags and sealed. About 6 or 7 pieces weighing approximately 170 g. were placed in each bag. One of these lots was frozen rapidly at –60°C. (–76°F.), using a mixture of alcohol and solid carbon dioxide. Bags were dipped into the freezing mixture until frozen. The average time for complete freezing was found to be 15 minutes. They were then stored at –20°C. (–4°F.). The other lots were allowed to freeze in still air at –4°C. (24.8°F.), –6°C. (21.2°F.) and –20°C. (–4°F.) and stored at the same respective temperatures.

Duplicate samples from each lot were taken out, allowed to thaw at room temperature and ground two times through a cold meat grinder. Thirty g. of this ground cod muscle were extracted for 45 seconds in a cold Turmix blender (similar to the Waring blender) using a baffle plate to avoid foaming as suggested by Dyer *et al. (4)*. Six hundred ml. of 5% sodium chloride solution at 0°C. containing 0.02 M sodium bicarbonate to give a pH of 7.0 to 7.5 were used to extract muscle proteins *(4)*.

We could not prevent foam formation effectively by the use of a baffle plate as

recommended by Dyer *et al. (4)*. These authors have recommended a blending time of 3-5 minutes. We found, however, that if such a long blending time was used, the rise of temperature of the blended mixture was more than 10°C. (18°F.) and an appreciable amount of air was dispersed in it. By blending for 45 seconds, good dispersion of the muscle tissue was obtained, and the foam formed soon rose to the top of the liquid on standing. More than 95% of the muscle tissue went into solution in this way.

The estimation of sulfhydryl groups was carried out by the ferricyanide method *(1, 12)* and also by oxidation with o-iodosobenzoic acid *(8, 9)*. The detailed procedure was as follows:

Ferricyanide method: To 10 ml. of the muscle extract 1 ml. of 1 M phosphate buffer (pH 7) was added followed by 1 ml. of 0.1 M potassium ferricyanide and the mixture was allowed to stand for half an hour. Twenty ml. of tungstic acid (10 ml. of 10% sodium tungstate plus 10 ml. of 1 N sulfuric acid plus 80 ml. distilled water) were then added to precipitate proteins. This was followed by 10 ml. of 1 N sulfuric acid and the volume made up to 50 ml. and filtered. To 5 ml. of the filtrate were added 0.1 ml. of 0.1 M potassium ferricyanide, 1 ml. of the ferric sulfate reagent *(6)* followed by 10 ml. of distilled water. The colour was then measured after half an hour at 710 mμ using a Beckman quartz spectrophotometer.

For denaturing proteins 5 ml. of 6% Duponol PC solution was added to 10 ml. of the muscle extract after the addition of phosphate buffer and potassium ferricyanide. The rest of the procedure was exactly the same.

o-Iodosobenzoic acid method: Fifty ml. of the above muscle extract were placed in a 400-ml. beaker to which 20 ml. of 0.1 M phosphate buffer (pH 7) were added, followed by 5.0 ml. of standardised, 0.009 N o-iodosobenzoic acid. The mixture was gently stirred and kept for 3 minutes. During this time a flask containing 5.0 ml. of standardised, freshly diluted 0.01 N sodium thiosulfate, 5 ml. of freshly prepared 4% potassium iodide and 6 ml. of 1 N hydrochloric acid (or enough to give a final pH of 1.5 to 2.0) was prepared. The contents of this flask were transferred quantitatively into the beaker and the volume made to about 200 ml. with distilled water. With constant stirring, the mixture was titrated electrometrically with 0.009 N o-iodosobenzoic acid using the "dead stop" end-point technique of Foulk and Bawden *(7, 14)*. At the end point the presence of free iodine caused a slight permanent deflection of the galvanometer.

For denaturing proteins to 50 ml. of the muscle extract, 20 ml. of 0.1 M phosphate buffer were added (pH 7) and 1 g. of Duponol PC which was dissolved separately in a small amount of water and then added to the muscle extract. This was allowed to stand for half an hour under nitrogen. The rest of the procedure was exactly the same.

Total nitrogen was determined on a 10-ml. aliquot of the muscle extract by the method of Kjeldahl-Lundin-Ellburg *(11)*. To determine soluble nitrogen the extract was centrifuged for 25 minutes at 5°C. (41°F.) and at 1000 r.p.m. Ten ml. aliquot of the clear supernatant liquid was taken for nitrogen determination by the same method *(11)*.

RESULTS AND DISCUSSION

The first part of this investigation was conducted on the white of eggs to find out whether some relationship existed between the "masked" sulfhydryl groups and freezing denaturation of proteins of egg white as measured by the decrease in the amount of soluble nitrogen. The determination of sulfhydryl groups was made by amperometric titration with silver nitrate *(2, 10)*. The data are presented in Table 1 where the difference in the amount of sulfhydryl groups of denatured and native egg white (the so-called "masked" sulfhydryl groups) and water soluble nitrogen is given. Each figure represents a mean of duplicate determinations.

TABLE 1

"Masked" sulfhydryl groups and water soluble nitrogen of egg white stored at 0°C. and frozen and stored at −5°C., −10°C., −15°C. and −20°C.

Days	0°C. (32°F.) (unfrozen)		−5°C. (23°F.)		−10°C. (14°F.)		−15°C. (5°F.)		−20°C. (−4°F.)	
	Sulfhydryl groups	Soluble N	Sulfhydryl groups	Soluble N	Sulfhydryl groups	Soluble N	Sulfhydryl groups	Soluble N	Sulfhydryl groups	Soluble N
	mg. cysteine per 100 g. egg white	g. N per 100 g. egg white	mg. cysteine per 100 g. egg white	g. N per 100 g. egg white	mg. cysteine per 100 g. egg white	g. N per 100 g. egg white	mg. cysteine per 100 g. egg white	g. N per 100 g. egg white	mg. cysteine per 100 g. egg white	g. N per 100 g. egg white
0	87.6	1.42	87.6	1.42	87.6	1.42	87.6	1.42	87.6	1.42
6	42.1	1.35	89.2	1.25	85.1	1.21	42.6	1.55	61.7	1.51
11	71.7	1.32	66.2	1.42	85.3	1.50	50.5	1.53	63.2	1.38
28	18.6	1.66	42.3	1.57	45.5	1.56	55.0	1.54	53.8	1.54
48	51.8	1.26	55.7	1.43	53.1	1.35	58.3	1.36	62.7	1.38
70	58.6	1.09	54.6	1.30	49.1	1.34	52.8	1.23	61.7	1.29
97	48.1	0.81	58.7	0.79	49.5	0.87	55.2	0.88	62.4	0.89
117	44.7	1.49	57.5	1.58	51.9	1.47	45.9	1.39	61.8	1.40
134	43.1	1.40	44.0	1.44	55.0	1.41	58.1	1.65	.....	.....

TABLE 2

"Masked" sulfhydryl groups and nitrogen soluble in 5% NaCl of cod muscle stored frozen at different temperatures.

Days	−4°C. (24.8°F.)			−6°C. (21.2°F.)			−20°C. (−4°F.) (slow frozen)			−20°C. (−4°F.) (quick frozen)		
	Sulfhydryl groups			Sulfhydryl groups			Sulfhydryl groups			Sulfhydryl groups		
	Ferricyanide method mg. cysteine per 100 g. cod	o-Iodosobenzoic acid method mg. cysteine per 100 g. cod	Soluble N % of total N	Ferricyanide method mg. cysteine per 100 g. cod	o-Iodosobenzoic acid method mg. cysteine per 100 g. cod	Soluble N % of total N	Ferricyanide method mg. cysteine per 100 g. cod	o-Iodosobenzoic acid method mg. cysteine per 100 g. cod	Soluble N % of total N	Ferricyanide method mg. cysteine per 100 g. cod	o-Iodosobenzoic acid method mg. cysteine per 100 g. cod	Soluble N % of total N
0	73.3	59.1	96.6	69.1	61.6	97.8	68.4	50.8	96.2	62.0	50.9	98.5
6	70.7	67.0	103.8	66.2	59.3	88.3	69.3	35.9	106.1	39.5	46.2	81.8
21	58.5	54.2	39.6	71.5	58.7	42.3	70.1	29.8	70.3	63.1	50.0	72.1
50	11.5	8.9	40.1	5.9	4.9	30.7	52.8	17.4	77.5	41.1	41.7	84.4
72	54.0	43.9	36.9	45.2	35.4	39.3	67.0	33.4	79.0	45.7	50.0	87.9
92	42.0	38.1	36.7	69.9	56.9	36.7	76.9	49.8	66.6	57.2	53.5	78.3
112	24.7	26.8	31.2	57.8	32.2	32.2	48.9	42.5	57.0	44.5	38.3	52.0
148	.....	50.0	35.9	.....	50.5	30.3	.....	37.8	50.0	.....	30.7	42.3

A study of Table 1 shows that there is no continuous decrease either in the amount of "masked" sulfhydryl groups or in the amount of soluble nitrogen during the storage period at any of the temperatures. Theoretically speaking the amount of soluble nitrogen should decrease continuously with the progressive denaturation of proteins. This is not, however, the case. The behaviour of "masked" sulfhydryl groups and of soluble nitrogen can best be studied from Figures 1 and 2. It can be seen from Figure 1 that at storage temperatures of 0°C. (32°F.), –5°C. (23°F.), –10°C. (14°F.) and –20°C. (–4°F.) the value of "masked" sulfhydryl groups shows a sharp decrease and reaches a minimum value after 28 days, whereafter it begins to rise again and after 48 days becomes more or less constant, with the exception of 0°C. (32°F.), where after 48 days the value of "masked" sulfhydryl groups shows again a gradual decline. In the case of –15°C. (5°F.) the value of "masked" sulfhydryl groups falls sharply, reaching a minimum after 6 days; then it rises gradually and finally becomes constant throughout the rest of the experiment.

The effect of storage temperature is noteworthy. The higher the storage temperature the greater is the decrease in the amount of "masked" sulfhydryl groups during the first 28 days of the storage period. In other words, after 28 days of storage, "masked" sulfhydryl groups reach a minimum value which is lowest in the case of 0°C. (32°F.) and is successively higher at –5°C. (23°F.), –10°C. (14°F.) and –20°C. (–4°F.). At the storage temperature of –15°C. (5°F.) the minimum value attained after 6 days of storage is almost at the same level as that of –5°C. (23°F.) after 28 days. The effect of temperature on the value of "masked" sulfhydryl groups is not very signficant after 48 days of storage.

The variation in the amount of water soluble nitrogen of egg white during storage at 0°C. (32°F.), –5°C. (23°F.), –10°C. (14°F.), –15°C. (5°F.) and –20°C. (–4°F.) is recorded in Figure 2. At storage tem-

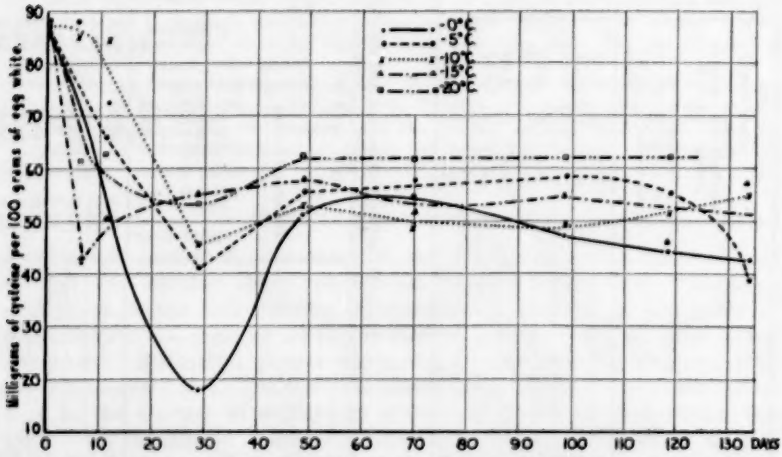


Figure 1. Variation of "masked" sulfhydryl groups of egg white stored at 0° and frozen stored at –5°, –10°, –15° and –20°C.

peratures of 0°C. (32°F.), –5°C. (23°F.), –10°C. (14°F.) and –20°C. (–4°F.) the value of water soluble nitrogen shows a slight decrease during the first 11 days of storage, rises and reaches a maximum after 28 days and then gradually begins to fall again. After 97 days, the value of water soluble nitrogen reaches a minimum and then shows a sharp rise in all the cases, attaining, after 117 days of storage, approximately the same value as at the start of the experiment. At a storage temperature of –15°C. (5°F.) water soluble nitrogen rises slightly during the first 6 days of storage and then shows the same pattern as in the case of other storage temperatures.

The effect of temperature is not so pronounced in the case of water soluble nitrogen as it is on the value of "masked" sulfhydryl groups.

Statistical analysis of the duplicate data indicates practically no correlation between the amount of "masked" sulfhydryl groups and water soluble nitrogen at any of the storage temperatures. This can also be seen from a comparison of the graphs presented in Figures 1 and 2.

The second part of this investigation was conducted on fresh cod fillets, which were frozen at –4°C. (24.8°F.), –6°C. (21.2°F.) and –20°C. (–4°F.) and stored at the same respective temperatures. A separate lot was also frozen rapidly at –60°C. (–76°F.) and stored at –20°C. (–4°F.). Two different methods, namely, the ferricyanide method *(1, 12)* and the o-iodosobenzoic acid method *(8, 9)*, were used for the estimation of sulfhydryl groups. The values of "masked" sulfhydryl groups as determined

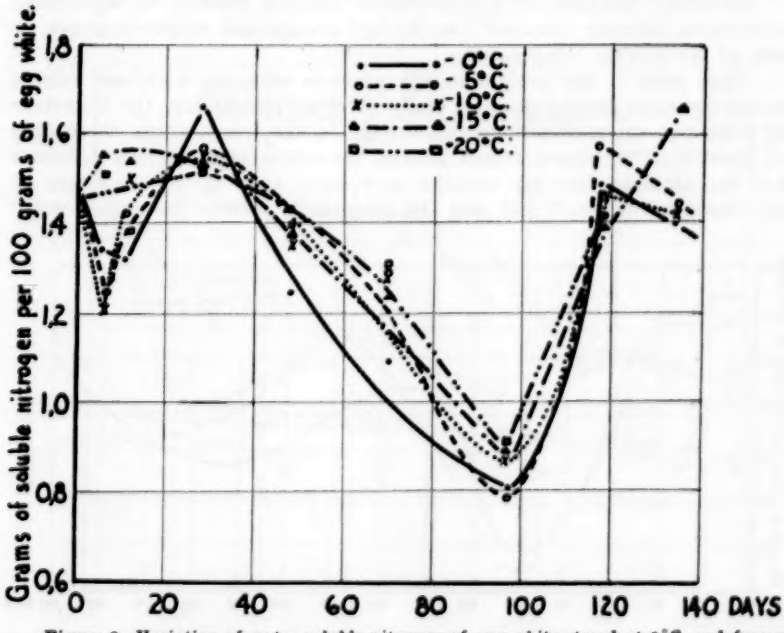


Figure 2. Variation of water soluble nitrogen of egg white stored at 0°C. and frozen stored at –5°, –10°, –15° and –20°C.

by these two methods and the percentage of soluble nitrogen are given in Table 2. Each figure represents a mean of duplicate determinations.

It can be seen from Table 2 that except for the fact that the values of "masked" sulfhydryl groups by the o-iodosobenzoic acid method are somewhat lower than by the ferricyanide method, these values of "masked" sulfhydryl groups by the two methods run parallel to each other. After 148 days of storage "masked" sulfhydryl groups could not be determined by the ferricyanide method due to turbidity of the solutions. The changes of "masked" sulfhydryl groups at various intervals of storage period as determined by the o-iodosobenzoic acid method are presented in Figure 3. A similar curve is obtained by plotting the data obtained by the ferricyanide method. At all the storage temperatures "masked" sulfhydryl groups reach a minimum value after 50 days of storage. Again the effect of storage temperature is noteworthy. The higher the storage temperature, the lower the value of this minimum.

Figure 4 indicates the variation of soluble nitrogen of cod muscle during frozen storage at all the temperatures studied. During the first 21 days of storage at –4°C. (24.8°F.) and –6°C. (21.2°F.) there is a sharp decrease in the amount of soluble nitrogen reaching a value of about 40% and then there is a gradual decline during the rest of the storage period. In the case of storage at –20°C. (–4°F.), both slow and quick frozen, soluble nitrogen falls to about 70% during the first 21 days of storage, rises slightly and then begins to decline again.

Statistical analysis of the duplicate samples showed no significant correlation between "masked" sulfhydryl groups and soluble nitrogen at any of the storage temperatures.

Since most of the protein in egg white is albumin, while cod muscle mostly contains actomyosin, our study gives an insight into the behaviour of these two types of proteins on storage in the frozen state. According to Dyer *(3)*, "in stored frozen muscle, the actomyosin fraction decreases but the albumin fraction remains unchanged even up to conditions of extreme denaturation" and that the decrease in soluble protein extracted

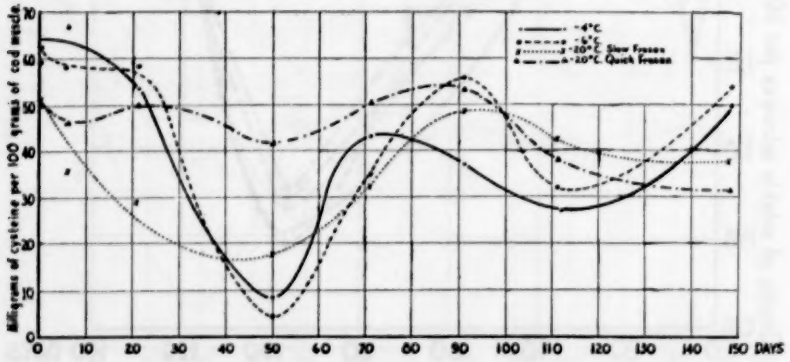


Figure 3. Variation of "masked" sulfhydryl groups of cod muscle stored frozen at different temperatures. (o-Iodosobenzoic acid method.)

after frozen storage "occurs in the actomyosin fraction, which gradually is reduced to zero as time of storage is lengthened." In the light of his investigations it can be stated that in our experiments with egg white there is little or no denaturation on frozen storage, and hence there is no continuous decrease in water soluble nitrogen (Fig. 2). This variation in the value of water soluble nitrogen of egg white is of interest in view of the observations of Evans *et al. (5)* who found an increase in the water insoluble protein fraction of egg albumin during the first 9 months of cold storage followed by a decrease. Mitchell *(13)* obtained an increase in water soluble nitrogen during 2 months of cold storage of shell eggs. In the case of cod muscle, on the other hand, soluble nitrogen shows, except for a slight rise after 21 days of storage in the case of -20°C. (-4°F.) (both slow and quick frozen), a steady decrease throughout the storage period (Fig. 4). As most of the protein in cod muscle is actomyosin, it can be stated that this decrease in soluble nitrogen occurred in the actomyosin fraction. This is in agreement with the observations of Dyer *(3)*.

A comparison of Figure 1 with Figure 3 shows a similarity of the changes in the value of "masked" sulfhydryl groups of both egg white and cod during the first part of the storage period. In both cases and at all the storage temperatures, "masked" sulfhydryl groups reach a minimum value after a certain period of storage and then begin to rise. On the basis of this similarity it can be stated that sulfhydryl groups underwent the same types of changes in egg white as they did in cod muscle.

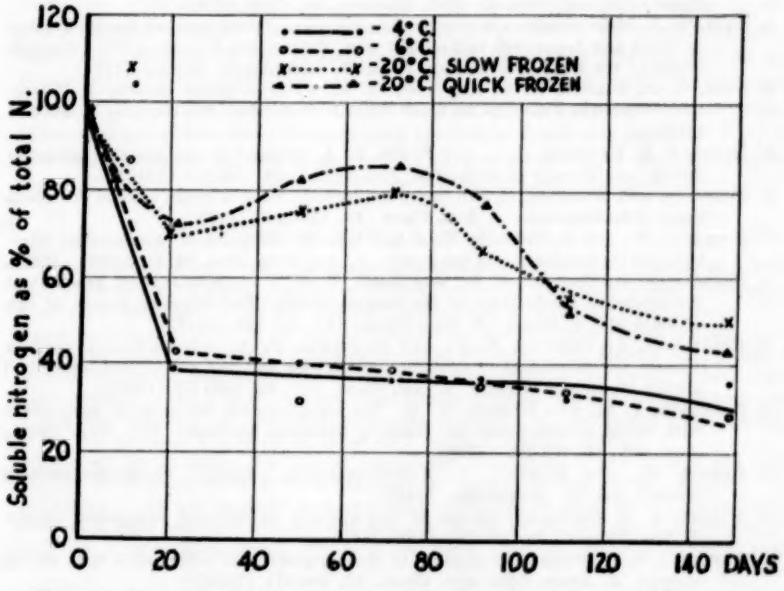


Figure 4. Percent of soluble nitrogen of cod muscle stored frozen at different temperatures.

SUMMARY

Egg white was frozen and stored at –5°C. (23°F.), –10°C. (14°F.), –15°C. (5°F.) and –20°C. (–4°F.). A separate lot was also stored at 0°C. (32°F.) (unfrozen). Water soluble nitrogen and sulfhydryl groups both before and after denaturation of proteins were determined at various intervals of the storage period. The difference between the total value of sulfhydryl groups as determined after denaturation of proteins and the free sulfhydryl groups was taken as an index of "masked" sulfhydryl groups. No correlation was observed between "masked" sulfhydryl groups and water soluble nitrogen.

Fresh cod fillets were frozen at –4°C. (24.8°F.), –6°C. (21.2°F.) and –20°C. (–4°F.) and stored at the same respective temperatures. A separate lot was frozen at –60°C. (–76°F.) and stored at –20°C. (–4°F.). No correlation existed between "masked" sulfhydryl groups and nitrogen soluble in 5% sodium chloride solution as determined at various intervals of the storage period.

Acknowledgment

The authors are indebted to Mr. L. Swenne for his help in adjusting the sensitivity of the galvanometer and the rotating platinum electrode.

LITERATURE CITED

1. Anson, M. L. The reactions of denatured egg albumin with ferricyanide. *J. Gen. Physiol.*, **23**, 247-261 (1939-40).
2. Benesch, R., and Benesch, R. E. Amperometric titration of sulfhydryl groups in amino acids and proteins. *Arch. Biochem.*, **19**, 35-45 (1948).
3. Dyer, W. J. Main problems of protein denaturation. *Proceedings* of the symposium on cured and frozen fish technology. SIK Publication *No. 100*, : VII. Swedish Institute for Food Preservation Research, Gothenburg, Sweden (1954).
4. Dyer, W. J., French, H. V., and Snow, J. M. Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. *J. Fish. Res. Bd. Canada*, **7**, 585-593 (1950).
5. Evans, R. J., Davidson, J. A., and Butts, H. A. Changes in egg proteins occurring during cold storage of shell eggs. *Poultry Sci.*, **28**, 206-214 (1949).
6. Folin, O., and Malmros, H. An improved form of Folin's micro method for blood sugar determinations. *J. Biol. Chem.*, **83**, 115-120 (1929).
7. Foulk, C. W., and Bawden, A. T. A new type of end point in electrometric titration and its application to iodometry. *J. Am. Chem. Soc.*, **48**, 2045-2051 (1926).
8. Hellerman, L., Chinard, F. P., and Dietz, V. R. Protein sulfhydryl groups and the reversible inactivation of the enzyme urease. The reducing groups of egg albumin and of urease. *J. Biol. Chem.*, **147**, 443-462 (1943).
9. Hellerman, L., Chinard, F. P., and Ramsdell, P. A. o-Iodosobenzoic acid, a reagent for estimation of cysteine, glutathione and the substituted sulfhydryl groups of certain proteins. *J. Am. Chem. Soc.*, **63**, 2551-53 (1941).
10. Kolthoff, I. M., and Harris, W. E. The amperometric titration of mercaptans with silver nitrate using the rotating platinum electrode. *Ind. Eng. Chem., Anal. Ed.*, **18**, 161-162 (1946).
11. Lundin, H., and Ellburg, J. Kvävebestämning. Kungl. Lantbruksstyrelsens Kungör n:r 14. Stockholm. (1932).
12. Mirsky, A. E. Sulfhydryl groups of egg albumin in different denaturing agents. *J. Gen. Physiol.*, **24**, 709-723 (1940-41).
13. Mitchell, L. C. Progressive changes in the composition of edible shell eggs during storage. *J. Assoc. Offic. Agr. Chem.*, **17**, 506-511 (1934).
14. Stock, J. T. The microchemical aspects of the "dead-stop" end-point titration method. *Metallurgia* (the British journal of metals), **37**, 220-223 (1947-48).

EFFECT OF FREEZING AND FREEZER STORAGE ON CAKE QUALITY. I. BAKED SPICE CAKES AND CAKES BAKED FROM FROZEN BATTERS

DORIS M. SKARHA AND FRANCES O. VAN DUYNE

Department of Home Economics, University of Illinois, Urbana, Illinois

(Manuscript received January 25, 1955)

Although studies on the effects of freezing and freezer storage on white, plain, and chocolate shortened cakes and on angel and sponge cakes have been published, little information on spice cakes is available. It has been stated that spice cake batters rather than baked spice cakes should be frozen and storage periods of 4 to 6 months for the frozen batters have been suggested *(8)*. However, no reports of controlled experiments to evaluate this type of cake were found in the literature and therefore the quality of freshly baked spice cakes compared with that of (a) thawed frozen spice cakes and (b) cakes baked from thawed frozen spice batters after selected periods of freezer storage became of interest. Objective measurements of index to volume and compressibility and subjective ratings of palatability were used as criteria of quality. Because recommendations for the packaging of batters for freezing and subsequent thawing and for the thawing of baked cakes vary, comparisons of three methods of packaging and thawing batters and two ways of thawing frozen baked cakes were also made after one interval of freezer storage. The results of this study should provide information on the best method of preparing spice cakes for freezing, on the advisable duration of freezer storage and on the flavor stability of spices (cinnamon, cloves, and nutmeg) when batters and baked cakes were frozen and stored. The latter point is of particular interest since spices used as seasoning in cooked foods are reported *(10)* to lose their potency during long periods of freezer storage. Some cloves, it should be mentioned, became stronger during freezer storage *(8)*.

EXPERIMENTAL PROCEDURE

The first series of spice cake batters was prepared on 12 days between March 11 and April 1, 1953. Three mixings were made each day and each mixing provided a fresh cake (standard 1) for testing and a batter and a baked cake for freezing. The frozen batters and baked cakes were designated for removal from freezer storage after 6 time intervals at random. The second series of spice cake batters was prepared between March 31 and November 10, 1953 on the days that the stored frozen cakes and batters of series 1 were removed from freezer storage. On each of these days one mixing was prepared from which a fresh cake (standard 2) was baked, while the rest of the mix provided 2 aliquots of batter or 2 baked cakes for freezing.

Preparation of cakes. Two lots of ingredients, with the exception of milk and eggs which were obtained prior to each baking, were used. The first lot was sufficient for the mixing of batters in series 1, the second for the batters in series 2. The formula, based on a spice variation of a two-egg cake recipe, was as follows: 254 g. hydrogenated fat,* 540 g. granulated sugar, 262 g. mixed whole eggs, 440 g. milk, 518.4 g. cake

* Crisco.

flour, 25.7 g. SAS-phosphate baking powder,[b] 4.1 g. salt, 6.5 g. cinnamon, and 1.6 g. each of cloves and nutmeg. The batters were mixed with an electric mixer[c] by a conventional method described by Lowe *(4)* in which the sifted dry ingredients and milk were added all at once to the creamed mixture.

Six-hundred grams of batter were weighed into aluminum baking pans, $8 \times 8 \times 2$ in., and baked in a preheated gas oven at 350°F. for 37 minutes. After baking, cakes were cooled at room temperature for 90 minutes (15 minutes in pan, and 75 minutes on rack) and held an additional 90 minutes at room temperature wrapped in wax-paper before testing.

Freezing, freezer storage, and preparation for testing. Methods used for packaging and thawing the batters and cakes in both series 1 and 2 are described in Table 1. In series 2, when batters were frozen as such, 6 replications were packed and thawed by methods a and b, b and c, and a and c in rotation.

The batters and cakes were frozen in a chest-type home freezer[d] where the temperature averaged 12.4° ± 1.4°F. during the experimental period. The frozen batters and cakes of series 1 were stored for 1, 4, 8, 16, 24 and 32 weeks, and after all intervals 6 of each were removed from freezer storage. The frozen batters and baked cakes of series 2 were held in storage for 1 and 4 weeks, respectively, and 12 replicates of each variation were prepared and tested. All of the thawed frozen batters were baked and cooled as the fresh cake batters were. Frozen baked cakes thawed at 300°F. were also cooled and held 3 hours before testing while the frozen baked cakes, thawed at room temperature, were tested 5 hours after removal from the freezer.

Quality testing. Index to volume and compressibility determinations and palatability ratings were made on halves of all cakes. Objective and subjective measurements were carried out as described by Owen and Van Duyne *(9)*. To determine index to volume, the area of the cut surface of a cake at the center is measured while the compressibility values represent the force required to compress slices of cake .75 inch in thickness to .50 inch at a uniform rate. For subjective measurements, 4 judges scored characteristics of the cakes on a 5-point scale. These ratings were weighted and added to give total palatability scores. In addition, the general desirability of all cakes was rated on a 5-point scale. Peroxide determinations were made on a fresh cake, a thawed frozen baked cake and a cake baked from a frozen batter after all storage intervals except the 1-week period. For these determinations, a quarter of a cake was finely crumbled and mixed; two 25-gram portions of the crumbs of each cake were allowed to air-dry overnight. The lipids were extracted from the crumbs by the procedure of Watts and Peng *(11)* and peroxide values of the filtrates were determined according to the procedure of Wheeler *(12)* as outlined by Jacobs *(3)*.

RESULTS AND DISCUSSION

Mean values and standard deviations of the means for index to volume and compressibility measurements of standard 1 and 2 cakes, of thawed frozen baked cakes and cakes baked from frozen batters after 6 intervals of freezer storage, are given in Table 2 while comparable figures for palatability ratings are presented in Table 3. Mean ratings for general desirability are not given because there was excellent correlation between them and the total palatability scores. Data for the peroxide determinations are not included since the titers for the lipid extracts did not exceed those of the blanks. The mean values for index to volume and total palatability scores of standard 2 cakes which were prepared on the days that the frozen products were removed from storage are, in some instances, signifi-

[b] Calumet.
[c] Sunbeam.
[d] Bishop.

TABLE 1

Packaging and thawing methods for spice cake batters and for baked spice cakes.

Series	State	Methods of packaging		Methods of thawing
		For freezing	For freezer storage	
1	Batter	Approximately 700 g. of batter in short, cylindrical carton with inner liner	As for freezing	2 hours at room temperature in carton[1]
	Baked cake	Cooled 3 hours at room temperature; heat-sealed in moisture-proof cellophane	Overwrapped with white paper using drug-store fold	30 minutes at 300° F. in cellophane wrapping
2	Batter	a. Approximately 700 g. of batter in short, cylindrical carton with inner liner	As for freezing	2 hours at room temperature in carton
	Batter	b. 600 g. of batter in pan; pan and batter heat-sealed in moisture-proof cellophane	As for freezing	30 minutes at room temperature in cellophane wrapping
	Batter	c. 600 g. of batter in wax-paper liner in pan	Liner and batter heat-sealed in moisture-proof cellophane	30 minutes at room temperature with cellophane wrapping removed
2	Baked cake	Cooled 3 hours at room temperature; heat-sealed in moisture-proof cellophane	Overwrapped with white paper using drug-store fold	5 hours at room temperature in cellophane wrapping
	Baked cake	Cooled 3 hours at room temperature; heat-sealed in moisture-proof cellophane	Overwrapped with white paper using drug-store fold	30 minutes at 300° F. in cellophane wrapping

[1] With the exception of batters removed after one week of freezer storage which were thawed overnight in a refrigerator and for two additional hours at room temperature.

TABLE 2

Effect of freezing and freezer storage on volume and compressibility of cakes.

Mean values for index to volume measurements and standard deviations of the means

Standard 1 cakes[1]	Standard 2 cakes[2]	Intervals of freezer storage	Thawed frozen baked cakes	Cakes baked from frozen batters
sq. in.	*sq. in.*	*wk.*	*sq. in.*	*sq. in.*
16.74 ± 0.142	16.71 ± 0.053	1	15.98 ± 0.092[3,4]	14.71 ± 0.137[3,4,6]
16.76 ± 0.048	16.72 ± 0.138	4	16.11 ± 0.104[3,4]	14.91 ± 0.067[3,4,6]
16.70 ± 0.078	16.20 ± 0.112[3]	8	15.55 ± 0.112[3,4]	15.31 ± 0.059[3,4]
16.88 ± 0.079	15.75 ± 0.138[3]	16	15.37 ± 0.088[3]	14.18 ± 0.123[3,4,6]
16.74 ± 0.048	16.04 ± 0.119[3]	24	14.27 ± 0.092[3,4,5]	14.45 ± 0.194[3,4]
16.83 ± 0.096	16.23 ± 0.098[3]	32	13.54 ± 0.150[3,4]	13.55 ± 0.204[3,4]

Mean values for compressibility measurements and standard deviations of the means

Standard 1 cakes[1]	Standard 2 cakes[2]	Intervals of freezer storage	Thawed frozen baked cakes	Cakes baked from frozen batters
g.	*g.*	*wk.*	*g.*	*g.*
54 ± 0.9	57 ± 0.8	1	54 ± 0.8	75 ± 1.3[3,4,6]
55 ± 1.1[7]	54 ± 0.9	4	54 ± 0.9	68 ± 1.1[3,4,6]
56 ± 0.9	51 ± 1.0[3]	8	52 ± 0.9[3]	63 ± 1.4[3,4,6]
55 ± 0.6	58 ± 1.5	16	46 ± 0.7[3,4]	69 ± 2.0[3,4,6,10]
58 ± 0.7	57 ± 1.1	24	64 ± 0.7[3,4,8]	70 ± 2.9[3,4,7]
59 ± 1.0	61 ± 0.8	32	73 ± 1.5[3,4,9]	81 ± 1.1[3,4,6,9]

[1] Standard 1 cakes baked on days cakes and batters were frozen.
[2] Standard 2 cakes baked on days frozen cakes and batters were tested.
[3] Significantly different from standard 1 cakes.
[4] Significantly different from standard 2 cakes.
[5] Mean value for 5 instead of 6 cakes.
[6] Significantly different from thawed frozen baked cakes.
[7] Mean value for 22 instead of 24 measurements.
[8] Mean value for 19 measurements on 5 cakes.
[9] Mean value for 21 measurements.
[10] Mean value for 23 measurements.

cantly* lower than the mean values of standard 1 cakes which were baked and tested on the days that cakes and batters were put into freezer storage. These differences are probably due to the fact that all the standard 1 cakes were prepared in March, whereas, the standard 2 cakes were prepared from March to November. However, the extent to which adverse conditions of temperature and humidity or changes in quality of ingredients were involved, is not known.

Effect of freezing and freezer storage on baked spice cakes and on spice cake batters. Mean values for index to volume measurements ranged from 15.75 to 16.88 sq. in. for standard cakes, from 13.54 to 16.11 sq. in. for thawed frozen baked cakes and from 13.55 to 15.31 sq. in. for cakes baked from frozen batters. With one exception, mean values for the standard cakes were significantly higher than mean values for either thawed frozen baked cakes or for cakes baked from frozen batters, and the mean index to volume measurements of thawed baked cakes were significantly greater than those of cakes baked from frozen batters after 1, 4 and 16 weeks of freezer storage. After 4 weeks of freezer storage, the frozen baked cakes showed a gradual decrease in volume until the 24-week interval when a marked decrease in volume was measured, while the cakes baked from

* A difference is considered significant when it is equal to or greater than three times the standard error of the difference between means.

TABLE 3

Effect of freezing and freezer storage on palatability of cakes.

	Number of cakes rated	Weeks of freezer storage	Number of ratings	Mean values for scores of characteristics of cakes								Mean total palatability scores and standard deviations of the means
				Shape	Surface	Volume	Texture	Grain	Color	Flavor	Freshness	
Maximum scores				10	10	10	20	10	10	20	10	100
Standard 1 cakes[1]	6		24	10	8	10	20	8	10	20	10	96 ± 0.3
Standard 2 cakes[2]	6		24	10	8	10	20	8	10	20	10	96 ± 0.5
Thawed frozen baked cakes	6	1	24	9	7	9	17	8	10	19	10	89 ± 0.9[3,4]
Cakes baked from frozen batters	6	1	24	7	5	7	14	6	9	17	8	73 ± 1.0[3,4,5]
Standard 1 cakes[1]	6		24	10	8	10	20	8	10	20	10	96 ± 0.3
Standard 2 cakes[2]	6		23	9	8	10	19	8	10	20	10	92 ± 1.0[3]
Thawed frozen baked cakes	6	4	24	9	7	9	18	8	10	18	9	87 ± 1.2[3,4]
Cakes baked from frozen batters	6	4	24	7	5	7	14	6	9	12	7	66 ± 1.2[3,4,5]
Standard 1 cakes[1]	6		24	10	8	10	20	8	10	20	10	96 ± 0.2
Standard 2 cakes[2]	6		24	9	8	10	19	8	10	19	9	91 ± 1.1[3]
Thawed frozen baked cakes	6	8	24	9	6	8	16	7	8	16	8	80 ± 1.5[3,4]
Cakes baked from frozen batters	6	8	24	7	5	8	13	5	8	9	7	62 ± 1.5[3,4,5]
Standard 1 cakes[1]	6		24	10	8	10	19	8	10	20	10	95 ± 0.5
Standard 2 cakes[2]	5		20	9	8	10	19	8	10	20	10	92 ± 1.0
Thawed frozen baked cakes	6	16	24	9	6	9	16	8	8	16	8	80 ± 1.1[3,4]
Cakes baked from frozen batters	6	16	24	6	4	6	13	5	7	10	7	59 ± 1.4[3,4,5]
Standard 1 cakes[1]	6		24	10	8	10	20	8	10	20	10	96 ± 0.4
Standard 2 cakes[2]	6		21	10	8	10	19	8	10	19	10	93 ± 0.9[3]
Thawed frozen baked cakes	5	24	18	7	6	7	11	6	8	12	6	63 ± 1.6[3,4]
Cakes baked from frozen batters	6	24	21	5	5	7	12	5	7	9	6	56 ± 2.2[3,4]
Standard 1 cakes[1]	6		24	10	8	10	20	8	10	20	10	95 ± 0.4
Standard 2 cakes[2]	6		24	10	8	10	19	8	10	20	10	94 ± 0.7
Thawed frozen baked cakes	6	32	24	6	5	6	9	6	7	12	4	55 ± 1.9[3,4]
Cakes baked from frozen batters	6	32	24	4	4	6	9	4	6	7	4	45 ± 1.9[3,4,5]

[1] Standard 1 cakes baked on days cakes and batters were frozen.
[2] Standard 2 cakes baked on days frozen cakes and batters were tested.
[3] Significantly different from standard 1 cakes.
[4] Significantly different from standard 2 cakes.
[5] Significantly different from thawed frozen baked cakes.

frozen batters were only considered to have satisfactory volume after 8 weeks of freezer storage. It is interesting that good agreement was obtained between means for the objective measurements and subjective ratings for volume. Graul and Lowe *(2)* and Mackey, Jones, and Dunn *(5)* have also observed that cakes baked from frozen batters were smaller in volume than standard and frozen baked cakes. On the other hand, Owen and Van Duyne *(9)* and Zaehringer and Mayfield *(13)* reported that plain cakes baked from batters frozen in cartons had slightly greater volumes and specific volumes, respectively, than frozen baked cakes.

The standard cakes had mean compressibility values ranging from 51 to 61 g., the thawed frozen baked cakes from 46 to 73 g. and the cakes baked from frozen batters from 63 to 81 g. The means for the cakes baked from frozen batters were significantly higher than those for the standard cakes or for the thawed frozen baked cakes with one exception. Thawed frozen baked cakes were more compressible than the standards after 16 weeks of freezer storage and less compressible after 24 and 32 weeks. When sodium-aluminum-sulfate baking powder was used in batters, Zaehringer and Mayfield *(13)* found no significant changes in compressibility of frozen baked cakes and cakes baked from batters frozen in cartons or pans during 4 months of freezer storage. Owen and Van Duyne *(9)* reported that the mean compressibility value of cakes baked from batters frozen in the pans and thawed before baking was significantly higher than the value for standard cakes.

Although the mean total palatability scores of thawed frozen baked cakes and of cakes baked from frozen batters are significantly lower than those of standard cakes (Table 3), the palatability scores of the thawed frozen baked cakes indicate that they were considered good after 1, 4, 8 and 16 weeks of freezer storage. The only characteristic which consistently received a mean rating corresponding to lower than good during this period was the surface of the cakes which was described as slightly soft and gummy. This effect has been noted by other workers *(1, 2, 13)*. After 24 weeks of freezer storage ratings of all individual characteristics except color were below good and the mean total palatability scores corresponded to fair. Further deterioration was observed in thawed frozen baked cakes after 32 weeks of freezer storage. Changes in flavor rather than development of off-flavors took place during 24 and 32 weeks of freezer storage. The following comments were made most frequently by the judges: presence of a stale spice flavor, lack of the blended flavor of spices present in the freshly baked standard cakes, or predominance of the flavor of cloves.

Mean palatability scores of cakes baked from frozen batters were significantly lower than those of thawed frozen baked cakes with the exception of cakes baked from frozen batters after 24 weeks of storage. When cakes baked from frozen batters after 1 week of freezer storage were only rated fair or below in the palatability factors of shape, surface, volume, texture and grain, the thawing time was changed from overnight in the refrigerator and 2 hours at room temperature to 2 hours at room temperature. However, ratings for the physical characteristics remained in the same range and the question arose as to whether the latter thawing period was long enough for batters packaged and frozen in cartons.

Cakes tasted after 4 weeks of freezer storage had developed an off-flavor, most frequently described as "fishy," which persisted in all cakes baked from frozen batters after the remaining storage periods. Owen and Van Duyne *(9)* observed a similar flavor in 3 cakes baked from frozen batters after 12 and 16 weeks of storage but, since these batters had been prepared on one day, it was felt that the eggs or milk may have been of poor quality on that particular day. Graul and Lowe *(2)*, Meyer, Buckley, and Moore *(6)* and Mackey, Jones, and Dunn *(5)* have reported off-flavor development usually after longer storage periods in plain, butter, and white cakes, respectively, baked from frozen batters. Meyer, Buckley, and Moore *(7)* found that shortened cakes containing hydrogenated vegetable oil only developed slight off-flavors after 9 months of freezer storage. Conceivably, the rapid development of off-flavor in this series of frozen spice batters might have been caused by the presence of spices in the two-egg batters, the quality of the ingredients, or the packaging material. The development of the "fishy" flavor was not accompanied by the formation of detectable peroxides.

Effect of three packaging and thawing methods on quality of cakes baked from frozen batters. Mean values for index to volume and compressibility determinations and for total palatability scores are given in Table 4 for cakes baked from batters in series 2 which were packaged and thawed according to procedures a, b and c. It is interesting that these methods of packaging and thawing batters did affect the quality of cakes baked from frozen batters after 1 week of freezer storage. The mean index to volume values and palatability scores of cakes baked from batters frozen in pans, removed after 24 hours and heat-sealed in cellophane, were significantly higher than those of cakes either heat-sealed in cellophane in pans and frozen, or frozen in cartons. In addition cakes baked from batters frozen in cartons and thawed 2 hours at room temperature were less compressible than the others. These results are in accordance with the recommendations of Fenton *(1)* and Miller, Kolshorn, and Wiegand *(8)*.

Effect of two thawing methods on quality of frozen baked cakes. Mean values for index to volume and compressibility measurements and palatability ratings for frozen baked cakes thawed after 4 weeks of freezer storage at room temperature for 5 hours do not differ significantly from those obtained for similar cakes thawed in a 300°F. oven for 30 minutes (Table 4). Graul and Lowe *(2)*, Meyer, Buckley, and Moore *(6)*, and Mackey, Jones, and Dunn *(5)* reported that frozen baked cakes were of higher quality than the cakes baked from frozen batters. All of these cakes were thawed at room temperature. The frozen baked cakes in the studies of Owen and Van Duyne *(9)* and Zaehringer and Mayfield *(13)* were thawed in a 300°F. oven for 30 and 15 minutes, respectively. The former *(9)* reported that thawed frozen baked cakes were lower in quality than cakes baked from the frozen batters; the latter *(13)* reported that the palatability of all the frozen products was similar to the freshly baked. Results of the present study indicate that the two methods of thawing used do not play an important role in determining the quality of thawed frozen baked spice cakes.

TABLE 4

Effect of variations in treatment of frozen spice batters and cakes on volume, compressibility and palatability.

Treatment of frozen batters or cakes	Intervals of freezer storage	Means and standard deviations of the means. Number of values in means are in parentheses.		
		Index to volume	Compressibility	Palatability
	wk.	*sq. in.*	*grams*	
Cakes baked from frozen batters packaged in cartons; thawed 2 hours at room temperature	1	14.84 ± 0.071 (36)	70 ± 1.2 (45)	72 ± 1.0 (47)
Cakes baked from frozen batters packaged in the pans and heat-sealed in cellophane; thawed 30 minutes at room temperature	1	15.15 ± 0.096[1] (36)	62 ± 0.8[1] (47)	79 ± 0.9[1] (47)
Cakes baked from frozen batter, frozen in the pans, removed and heat-sealed in cellophane; thawed 30 minutes at room temperature	1	15.84 ± 0.060[1,2] (36)	63 ± 0.9[1] (48)	84 ± 0.7[1,2] (46)
Frozen baked cakes; thawed 5 hours at room temperature	4	16.07 ± 0.067 (36)	50 ± 0.8 (46)	85 ± 1.1 (48)
Frozen baked cakes; thawed by reheating in a 300°F. oven for 30 minutes	4	15.93 ± 0.076 (36)	47 ± 0.7 (47)	84 ± 1.0 (48)

[1] Significantly different from cakes baked from frozen batters packaged in cartons.
[2] Significantly different from frozen batters packaged in pans and heat-sealed in cellophane.

SUMMARY

Products of better quality were obtained if spice cakes were baked before freezing than if frozen spice batters were baked. After freezing and 1, 4, 8, and 16 weeks of freezer storage, baked spice cakes, thawed 30 minutes in an oven, were considered good on the basis of objective measurements of index to volume and compressibility and subjective ratings of palatability. By the same criteria cakes baked from frozen spice batters packaged in cartons were not good even after 1 week of freezer storage. After 4 weeks of freezer storage off-flavors were noticeable in cakes baked from these batters.

When a series of spice batters was packaged for freezing in three ways and held in freezer storage 1 week, cakes baked from spice batters, frozen in wax-paper liners in baking pans, removed from pans after 24 hours, heat-sealed in moisture-proof cellophane, and thawed 30 minutes before baking were of better quality than cakes baked from batters frozen and stored in baking pans and cartons. Whether the improvement in physical characteristics resulted from the method of packaging or the amount of thawing and whether similar results would be obtained after longer intervals of freezer storage are not known.

When frozen baked spice cakes were thawed in the oven or at room temperature after 4 weeks of freezer storage, comparable mean values for

index to volume, compressibility and palatability were obtained, indicating that neither method was superior to the other for these cakes.

It is evident that further investigations on the effect of the nature and quality of the ingredients, of different methods of mixing and of various ways of packaging and thawing products on the quality of frozen baked cakes and cakes prepared from frozen batters are needed.

LITERATURE CITED

1. FENTON, F. Foods from the freezer: precooked and prepared. N. Y. Agr. Coll. (Cornell) Ext. *Bull. 692*, (Rev. 1951).
2. GRAUL, L. S., AND LOWE, B. How storage affects frozen cakes and batters. *Food Inds.*, **19**, 330 (1947).
3. JACOBS, M. B. *The Chemical Analysis of Foods and Food Products.* 1938, D. Van Nostrand Company, Inc., New York, N. Y.
4. LOWE, B. *Experimental Cookery. From the Chemical and Physical Standpoint.* 3rd ed., 1943, John Wiley and Sons, Inc., New York, N. Y.
5. MACKEY, A. O., JONES, P., AND DUNN, J. The effect of ingredient variations on the quality of white and chocolate cakes baked prior to freezing or baked from frozen batters. *Food Research*, **17**, 216 (1952).
6. MEYER, B., BUCKLEY, R., AND MOORE, R. Research shows differences in frozen butter cake and sponge cake. *Refrig. Eng.*, **57**, 340 (1949).
7. MEYER, B., MOORE, R., AND BUCKLEY, R. Flavor deterioration in frozen cake batters. *Food Research*, **18**, 70 (1953).
8. MILLER, R. C., KOLSHORN, A. M., AND WIEGAND, E. H. Freezing cooked and prepared foods. Ore. Sta. Coll. Agr. Exp. Sta. *Bull. 494* (1951).
9. OWEN, R. F., AND VAN DUYNE, F. O. Comparison of the quality of freshly baked cakes, thawed frozen baked cakes, and cakes prepared from batters which had been frozen. *Food Research*, **15**, 169 (1950).
10. TRESSLER, D. K., EVERS, C. F., AND LONG, L. *Into the Freezer and Out.* 1946, The Avi Publishing Company, Inc., New York, N. Y.
11. WATTS, B. M., AND PENG, D. H. Rancidity development in raw versus precooked frozen pork sausage. *J. Home Econ.*, **39**, 88 (1947).
12. WHEELER, D. H. Peroxide formation as a measure of autoxidative deterioration. *Oil and Soap*, **9**, 89 (1932).
13. ZAEHRINGER, M. V., AND MAYFIELD, H. L. The effect of leavening and shortening combinations on the frozen storage of cakes and cake batters prepared at high altitude. *Food Technol.*, **5**, 151 (1951).

EFFECT OF FREEZING AND FREEZER STORAGE ON CAKE QUALITY. II. VARIATION IN BATTER TREATMENT BEFORE AND AFTER FREEZER STORAGE

DORIS M. SKARHA AND FRANCES O. VAN DUYNE
Department of Home Economics, University of Illinois, Urbana, Illinois

(Manuscript received January 25, 1955)

Undesirable characteristics in cakes baked from frozen batters have been reported by numerous investigators *(1, 2, 3, 4, 9)*. Other workers *(5, 7, 10)* have found that cakes baked from stored frozen batters were similar to freshly baked cakes. In Part I of this study *(8)* the quality of spice cakes baked from batters which were frozen and stored in cartons and thawed 2 hours at room temperature before baking was found to be markedly inferior to that of thawed frozen baked spice cakes after 4 to 32 weeks of freezer storage. However, when a second series of batters was baked after only one week of freezer storage, better cakes were obtained from batters frozen in wax-paper liners in pans, removed and heat-sealed in cellophane for freezer storage, and thawed 30 minutes prior to baking than from batters packaged in pans heat-sealed in cellophane, frozen, stored, and thawed 30 minutes before baking or from batters frozen in cartons and thawed 2 hours at room temperature.

One objective of this investigation was to compare the effect of 3 packaging and thawing procedures on the quality of cakes baked from spice batters after 4 and 16 weeks of freezer storage. The other objective was to find out whether the composition of the cartons used or the inclusion of spices in the recipe caused the rather rapid development of off-flavor in cakes baked from thawed frozen spice batters reported in Part I *(8)*.

EXPERIMENTAL PROCEDURE

Ingredients were purchased and spice cake batters were prepared according to the procedures of Skarha and Van Duyne *(8)*. On 6 days between January 10 and January 21, 1954, 3 batters were mixed each day and each batter provided a fresh standard spice cake for testing and 2 portions of spice cake batter to be packaged for freezing and to be thawed in one of three ways. The following methods were used: a. 600 g. of batter frozen in wax-paper liner in pan, liner and batter heat-sealed in moisture-proof cellophane for storage, and cellophane removed and batter thawed 30 minutes at room temperature before baking; b. approximately 700 g. of batter frozen and stored in short, cylindrical cartons which had inner liners and thawed to 32°F. in the carton; and c. approximately 700 g. of batter frozen and stored in tall, cylindrical cartons without inner liners and thawed to 63°F. in the cartons before baking. These methods were selected because method a. had given the best cakes baked from frozen batters after 1 week of freezer storage *(8)*, and method c. had been used satisfactorily for the packaging and thawing of plain cake batters in an earlier study *(7)* in which cakes baked from thawed frozen batters were of good quality and in which the presence of off-flavors in cakes baked from thawed frozen batters was the exception rather than the rule. Method b. is similar to the procedure used when batters were thawed and baked for comparison with thawed frozen baked cakes after 6 intervals of freezer storage *(8)* with the modification that the thawing period was approximately half again as long.

For a second series spice and plain cake batters were prepared on 12 days between February 28 and March 17, 1954. Plain cake batters were similar in composition to the

spice cake batters *(8)* except that the spices—cinnamon, cloves and nutmeg—were omitted. Each day of mixing, a spice and a plain cake batter were prepared and from each batter a fresh standard cake was baked for testing, one portion of batter was treated according to method a. and one was frozen in a short, cylindrical carton with an inner liner and after freezer storage thawed to 63°F. before baking. The latter method combines the use of the more moisture-proof cartons and the more satifactory thawing procedure.

The temperature in the home freezer averaged $-11.2° \pm 1.8°$F. during the experimental period. Six replicates of each variation in both series were removed from freezer storage after 4 and 16 weeks. The thawed batters were baked, cooled, and tested for quality as before *(8)* except that 13 of the 36 batters, packaged in cellophane and thawed 30 minutes at room temperature required baking periods of 38 to 40 minutes instead of 37 minutes.

RESULTS AND DISCUSSION

During freezer storage no significant changes occurred in the weights of batters packaged in the short, cylindrical cartons with treated inner liners. However, after 16 weeks of freezer storage the mean weight loss for batters packaged in tall, cylindrical cartons without inner liners was 12 ± 1 gm. and crusts had formed along the edges on top of the batters. In an earlier study *(7)* there was no significant change in weight when batters were frozen and stored for 16 weeks in this type of container. Batters packaged in cellophane and held in freezer storage for 16 weeks had a mean weight loss of 15 ± 3 grams—largely accounted for by the presence of ice crystals between the cellophane and surface of the batters.

Mean thawing times for the cake batters were 181 ± 3.3 minutes for batters frozen in short, cylindrical cartons and thawed to 32°F., 277 ± 5.3 minutes for batters frozen in tall, cylindrical cartons and thawed to 63°F., and 270 ± 3.8 minutes for batters frozen in short, cylindrical cartons and thawed to 63°F. The majority of batters, packaged in cellophane and thawed 30 minutes at room temperature, attained a mean temperature of $30.2° \pm 1.8°$F.; however, due to an unusually hot June and July the spice and plain batters thawed after 16 weeks of freezer storage reached a mean temperature of $53.6° \pm 1.8°$F. in 30 minutes.

Effect of variations in packaging and thawing on quality of cakes baked from frozen spice batters. Mean values and standard deviations of the means for measurements of index to volume and compressibility and for total palatability scores are given in Table 1. After 4 weeks of freezer storage, only the cakes baked from batters frozen in tall, cylindrical cartons and thawed to 63°F. before baking (method c.) had volumes similar to the freshly baked standard cakes. The mean volumes of cakes baked from the other batter treatments were significantly lower than the means for the standard cakes. With all treatments there were decreases in volume between 4 and 16 weeks of freezer storage and the decrease was significant in the case of batters packaged in cellophane and thawed 30 minutes at room temperature. After 16 weeks of freezer storage, even the mean volume of cakes baked from batters packaged in tall, cylindrical cartons and thawed to 63°F. was significantly lower than that of the standards.

The compressibility of cakes baked from frozen batters was not affected as much by batter treatment as by increased length of freezer storage. Mean values for the compressibility of cakes baked from frozen batters were significantly higher than the means for standard cakes after both intervals

TABLE 1

Effect of variations in packaging and thawing on quality of cakes baked from frozen spice batters

	Treatment of batters			Weeks of freezer storage	Mean values and standard deviations of the means		
	Treatment designated as	Packaged in	Method of thawing		Index to volume	Compressibility	Palatability
					sq. in.	*g.*	
Standard cakes[1]					16.41 ± 0.113	57 ± 0.6	95 ± 0.6
Cakes baked from frozen batters	a	Heat-sealable cellophane	30 min. at room temp.	4	15.84 ± 0.057[2,3,4]	62 ± 0.7[2,3,5]	82 ± 0.9[2,3,5]
Cakes baked from frozen batters	a	Heat-sealable cellophane	30 min. at room temp.	16	14.46 ± 0.199[2,6,7]	78 ± 1.5[2,7]	53 ± 1.8[2,7,8]
Standard cakes[1]					16.51 ± 0.031	57 ± 0.7	95 ± 0.4
Cakes baked from frozen batters	b	Short cylindrical carton	At room temp. to 32°F.	4	15.37 ± 0.141[2,9]	72 ± 2.0[2]	68 ± 1.6[2,8,9]
Cakes baked from frozen batters	b	Short cylindrical carton	At room temp. to 32°F.	16	15.09 ± 0.250[2]	85 ± 2.0[2,7]	54 ± 2.2[2,7,8]
Standard cakes[1]					16.24 ± 0.039[10]	57 ± 0.6	96 ± 0.4
Cakes baked from frozen batters	c	Tall cylindrical carton	At room temp. to 63°F.	4	16.12 ± 0.074	66 ± 1.3[2]	79 ± 1.5[2,8]
Cakes baked from frozen batters	c	Tall cylindrical carton	At room temp. to 63°F.	16	15.73 ± 0.114[2]	79 ± 1.1[2,7]	58 ± 1.3[2,7,8]

[1] Standard cakes baked on days batters were frozen.
[2] Significantly different from mean value obtained for standard cakes.
[3] Significantly different from mean value obtained for treatment b after 4 weeks of freezer storage.
[4] Mean value for 5 instead of 6 cakes.
[5] Mean value for 19 measurements on 5 cakes instead of 24 on 6 cakes.
[6] Significantly different from mean value obtained for treatment c after 16 weeks of freezer storage.
[7] Significantly different from mean value for same treatment after 4 weeks of freezer storage.
[8] Mean value for 23 instead of 24 ratings.
[9] Significantly different from mean value obtained for treatment c after 4 weeks of freezer storage.
[10] Significantly different from mean value for standard cakes for treatment b.

of freezer storage; mean values for cakes prepared after 16 weeks of freezer storage were significantly higher than those of cakes baked after 4 weeks. Cakes baked from batters packaged in short, cylindrical cartons and thawed to 32°F. were significantly less compressible after 4 weeks of freezer storage than cakes baked from batters packaged in cellophane and thawed 30 minutes at room temperature. No significant differences in compressibility were observed between cakes prepared from 3 batter treatments after 16 weeks of freezer storage.

Mean palatability scores of cakes baked from thawed frozen batters were significantly lower after both intervals of freezer storage than the means obtained for the standard cakes, and the mean scores of cakes prepared after 16 weeks of freezer storage were significantly lower than those baked after 4 weeks. However, cakes baked from either batters packaged in cellophane and thawed 30 minutes at room temperature or batters frozen and stored in tall, cylindrical cartons and thawed to 63°F. before baking were significantly more palatable after 4 weeks of freezer storage than cakes baked from batters packaged in short, cylindrical cartons and thawed to 32°F. These cakes were rated lower on all palatability factors and significantly so on surface, texture, grain and color than were cakes baked after the two other treatments. But after 16 weeks of freezer storage there were no significant differences between the mean palatability scores of cakes prepared from the 3 batter treatments. At this time undesirable flavors, similar to those reported *(8)* were present in the cakes baked from all the frozen batters irrespective of the packaging material used. The mean scores for flavor of cakes baked from thawed frozen batters after 16 weeks of freezer storage were 10 of a possible 20 in the previous study and 9 or 10 in this series.

On the basis of volume and compressibility measurements and palatability ratings thawing batters frozen in cartons to 63°F. instead of 32°F. before baking gave cakes of better quality. Differences were more marked after 4 weeks of freezer storage than after 16 but the former method may have been penalized by the fact that the tall, cylindrical cartons were not found to be satisfactorily moisture-proof.

Comparison of spice and plain cakes baked from batters packaged for freezing and thawed by two methods. Mean values and standard deviations of the means for index to volume, compressibility and palatability of these cakes are presented in Table 2. Standard plain cakes were significantly, although only slightly, smaller in volume than standard spice cakes, but after 4 and 16 weeks of freezer storage there were no significant differences in mean volumes due to type of batter. All spice and plain cakes baked from thawed frozen batters had significantly smaller mean volumes than the standard cakes and the method of packaging and thawing the batters definitely affected the resulting volumes. After 16 weeks of freezer storage cakes of better volume were obtained if the batters were frozen in short, cylindrical moisture-proof cartons and thawed to 63°F. before baking than if packaged in cellophane and thawed 30 minutes at room temperature. The mean value for the latter cakes was lower in this series than in the previous one, perhaps because the batters attained a higher mean temperature during thawing.

TABLE 2

Comparison of spice and plain cakes baked from batters packaged for freezing and thawed by two methods

Quality factors	Mean values and standard deviations of the means			
	Standard cakes[1]	Cakes baked from batters held in freezer storage 4 weeks		
		Batters packaged in cellophane and thawed 30 min. at room temperature		Batters packaged in short cylindrical cartons and thawed to 63°F.
	Spice	Spice	Plain	Spice	Plain
Index to volume—sq. in.	16.50 ± 0.054	15.32 ± 0.199[2]	15.36 ± 0.149[2]	15.86 ± 0.095[2]	15.94 ± 0.043[2,4]
Compressibility—g.	57 ± 0.5[5]	66 ± 1.2[2,6]	65 ± 1.1[5,6]	66 ± 1.2[2]	66 ± 1.3[6]
Palatability	94 ± 0.6	71 ± 2.2[2,6]	82 ± 1.2[2,3,6]	67 ± 1.8[2,6]	82 ± 1.1[2,3,6]
	Cakes baked from batters held in freezer storage 16 weeks				
Index to volume—sq. in.	16.80 ± 0.048[7]	13.05 ± 0.305[2,7]	13.59 ± 0.186[3,7]	16.22 ± 0.062[2,4,7]	16.18 ± 0.076[2,4]
Compressibility—g.	56 ± 0.5[6]	88 ± 2.6[3,7]	87 ± 2.1[3,7]	63 ± 1.0[2,4,6]	65 ± 0.6[2,4]
Palatability	95 ± 0.4	51 ± 1.6[3,7]	63 ± 2.3[2,3,7]	73 ± 1.1[3,4]	81 ± 0.9[2,3,4]

[1] Standard cakes baked on days batters were frozen.
[2] Significantly different from mean value for corresponding spice cakes.
[3] Significantly different from mean value for corresponding standard cakes.
[4] Significantly different from mean value for corresponding cakes packaged in cellophane and thawed 30 min. at room temperature.
[5] Mean value for 23 instead of 24 measurements.
[6] Mean value for 22 instead of 24 measurements or ratings.
[7] Significantly different from mean value for corresponding cakes after 4 weeks of freezer storage.

No significant differences between the mean values for compressibility of spice and plain cakes were observed. Mean compressibility values for spice and plain cakes baked from batters packaged in cellophane and thawed 30 minutes at room temperature before baking were significantly higher after 16 weeks of freezer storage than the mean values for cakes baked from batters frozen and stored in short, cylindrical cartons and thawed to 63°F. After both intervals of freezer storage spice and plain cakes were less compressible than the standard cakes.

Standard spice and plain cakes were rated as being equally palatable. However, mean scores for plain cakes baked from batters packaged and thawed by 2 methods were significantly higher after 4 and 16 weeks of freezer storage than the mean scores of the corresponding spice cakes. Slightly lower ratings for several characteristics of palatability—texture, grain, color, flavor and freshness—accounted for the significant differences in the total palatability scores. Although the mean flavor scores for spice cakes were lower than for plain cakes, for the first time no "fishy" off-flavors were noticed in this series of cakes baked from thawed frozen spice batters. The flavor of the spice cakes baked from thawed frozen batters was either not commented upon or was described as being stale or soapy or having a different spice flavor. Cakes baked from frozen batters packaged in cellophane and thawed 30 minutes at room temperature did not differ significantly in palatability after 4 weeks of freezer storage from cakes baked from batters packaged and stored in moisture-proof cartons and thawed to 63°F. at room temperature before baking but were significantly less palatable after 16 weeks of freezer storage. On the other hand cakes baked from batters packaged in moisture-proof cartons and thawed to 63°F. before baking were of as good quality after 16 weeks of freezer storage as after four.

Skarha and Van Duyne *(8)* found that spice cakes baked from batters which had been frozen in wax-paper liners in pans, removed after 24 hours, heat-sealed in moisture-vapor-proof cellophane for storage, and thawed at room temperature for 30 minutes before baking were of good quality after 1 week of freezer storage. Results of this study show that it is a fairly satisfactory method for a period of freezer storage as long as 4 weeks but not for one as long as 16 weeks. For the latter length of storage a packaging method giving less batter surface in proportion to volume allowed for the preparation of higher quality cakes. Moore, Meyer, and Buckley *(6)* found that when cakes were frozen and stored at different temperatures there appeared to be a direct relationship between the amounts of carbon dioxide lost from the batters and the deterioration in quality of the cakes baked from the batters. As these authors suggested, it seems possible that when more batter surface is exposed during freezer storage and thawing more leavening action is lost.

Packaging material was ruled out as a cause of the off-flavor since "fishy" off-flavors were present in spice cakes baked from batters packaged in cellophane, in tall, cylindrical cartons, or in short, cylindrical cartons with inner liners in one comparison but not in the second when the batters were packaged in cellophane or in the latter type of carton. Furthermore, results obtained with this series of cakes in which a second lot of dry

ingredients was used showed that the inclusion of spices did not always result in the development of "fishy" off-flavors. Neither the plain nor the spice cakes had these off-flavors, although cakes baked from plain thawed batters were more palatable and had higher flavor ratings than cakes baked from thawed spice batters. At present it seems possible that the presence or absence of "fishy" off-flavors is determined by the composition, quality, or stability of one or more of the original ingredients including the spices.

SUMMARY

Spice cakes baked from batters frozen in wax-paper liners in pans, removed from pans after 24 hours, heat-sealed in moisture-proof cellophane for storage, and thawed 30 minutes at room temperature before baking were of satifactory quality as measured by index to volume and compressibility measurements and had mean palatability ratings corresponding to *good* and *high fair* after 4 weeks of freezer storage. However, after 16 weeks of freezer storage cakes baked from these batters were not acceptable.

The quality of cakes baked from batters packaged in cartons was affected by the moisture-proofness of the container and the temperature to which the batter was thawed before baking. When batters were packaged in moisture-proof cartons and thawed to 63°F. before baking, cakes were of *good* to *high fair* quality after both 4 and 16 weeks of freezer storage.

Cakes baked from plain batters after 4 and 16 weeks of freezer storage did not differ in volume and compressibility from cakes baked from spice batters but the palatability scores were significantly higher for the plain cakes. The "fishy" off-flavor present in some cakes baked from thawed frozen spice batters was not caused primarily by the packaging material nor by the inclusion of spices in the batters.

LITERATURE CITED

1. Graul, L. S., and Lowe, B. How storage affects frozen cakes and batters. *Food Inds.*, 19, 330 (1947).
2. Mackey, A. O., Jones, P., and Dunn, J. The effect of ingredient variations on the quality of white and chocolate cakes baked prior to freezing or baked from frozen batters. *Food Research*, 17, 216 (1952).
3. Meyer, B., Buckley, R., and Moore, R. Research shows differences in frozen butter cake and sponge cake. *Refrig. Eng.*, 57, 340 (1949).
4. Meyer, B., Moore, R., and Buckley, R. Flavor deterioration in frozen cake batters. *Food Research*, 18, 70 (1953).
5. Miller, C., and Beattie, I. E. On the freezing and frozen storage of cakes. *J. Home Econ.*, 41, 463 (1949).
6. Moore, R., Meyer, B., and Buckley, R. The effect of freezer storage temperatures on cake quality and on the carbon dioxide content of cake batters. *Food Research*, 19, 590 (1954).
7. Owen, R. F., and Van Duyne, F. O. Comparison of the quality of freshly baked cakes, thawed frozen baked cakes, and cakes prepared from batters which had been frozen. *Food Research*, 15, 169 (1950).
8. Skarha, D. M., and Van Duyne, F. O. Effect of freezing and freezer storage on cake quality. I. Baked spice cakes and cakes baked from frozen batters. *Food Research*, 20, 273 (1955).
9. Sunderlin, G. L., Collins, O. D., and Acheson, M. Frozen batters and doughs. *J. Home Econ.*, 32, 381 (1940).
10. Zahringer, M. V., and Mayfield, H. L. The effect of leavening and shortening combinations on the frozen storage of cakes and cake batters prepared at high altitude. *Food Technol.*, 5, 151 (1951).

A CHEMICAL STUDY OF THE PROGRESSIVE DEVELOPMENT OF OFF-FLAVOR IN FROZEN RAW VEGETABLES[a]

FRANK A. LEE, A. C. WAGENKNECHT, AND J. C. HENING[b]

New York State Agricultural Experiment Station, Cornell University, Geneva, N. Y.

(Manuscript received February 7, 1955)

It has been known for a long time that unblanched vegetables deteriorate in quality when held in frozen storage for extended periods of time. Rancidity of the lipid matter is one of the primary causes of off-flavor in unblanched peas which had been stored at 0°F. (–17.8°C.) *(5)*. Large increases in acid numbers together with increases in peroxide numbers were observed in the lipids extracted from raw peas after several years of frozen storage. The main increase in acid number occurred during the first year of storage *(8)*. Similar changes were noted in the lipids extracted from unblanched sweet corn, snap beans, and other vegetables after extended periods of storage at 0°F. (–17.8°C.) *(4)*.

In order to extend the knowledge of the changes which take place in such material, the present study was undertaken to determine the length of time that unblanched vegetables could be held in frozen storage before these undesirable changes could be detected, and to determine the course of the development of the acids and peroxides during the period of storage.

MATERIALS AND METHODS

Thomas Laxton peas, Wade snap beans, and Golden Cross sweet corn were grown on the Experiment Station farm and were harvested, processed, and stored, as previously described *(4, 5)*.

Quality changes were measured both chemically and by means of a taste panel. Samples from each lot were taken for immediate taste test at the time of harvest, and at regular intervals during storage, until obvious deterioration in quality was definitely established. At the same time, samples were dried by lyophilization and were extracted for 48 hours with peroxide-free anhydrous ethyl ether to obtain the crude lipid material, as described previously *(5)*. This work was started before it was determined that a 24-hour extraction period was sufficient to effect the extraction of the crude lipid *(4)*, but was continued on the 48-hour basis to keep the conditions uniform. The chemical determinations were continued at regular but lengthening intervals throughout the storage period. A final subjective and chemical examination of the 1952 samples was conducted after 2 years of storage. Chemical methods used were described previously *(4, 5)*.

In the 1952 series of experiments it seemed that the time intervals used were too long in the beginning, and, therefore, it was deemed wise to repeat the storage study with weekly intervals of sampling and taste tests, for about 6 weeks. These latter were run in 1954.

The vegetables were cooked according to the directions of Fenton *(2)*. The raw snap beans, however, were given an additional 3 minutes' cook to make the texture more comparable to that of the blanched vegetable. Also, it was found desirable to puree the snap beans after cooking but before serving them to the judges. This substantially eliminated texture differences which otherwise tended to influence the decision of the judges.

---

[a] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 990.

[b] Deceased February 17, 1955.

The taste tests were run in a darkened room so that differences in color would not be a factor.

The triangle taste test was chosen for comparative tests on the blanched and unblanched vegetables, to determine when off-flavors first developed in the unblanched samples and to follow progressive changes during storage. In these tests the taster was asked to match the like samples and score them using a 1-to-10 scale in which one was extremely low in quality and 10 was excellent *(1, 3, 7)*. The organoleptic tests were run by a panel of experienced judges.

RESULTS AND DISCUSSION

The increase in acids and the development of peroxides in the crude lipid extracted from peas, snap beans, and sweet corn are shown in Figures 1, 2, 3, 4, 5, and 6, and in Tables 1 and 2. Figures 1, 3, and 5 give the results obtained during the 2-year storage period. Figures 2, 4, and 6 show the results of the 6-week storage period at weekly intervals. Table 3 gives a summary of the taste tests.

In general, it was found that after a week some increase in acid was detected in the lipid extracted from the unblanched samples of all these vegetables, but the taste tests on the vegetables were inconclusive after this short period of storage. After 2 weeks, definite signs of rancidity or staleness were detected in the vegetables by the tasters, and a considerable increase in acid was found in the extracted lipid. After 2 weeks, color deterioration was observed visually in the raw snap beans. However, the peroxides did not appear in the extracted lipid until the end of the third week in peas, the fourth in snap beans, and after 2 months in sweet corn. Thus it seems that the peroxides started to develop in the unblanched

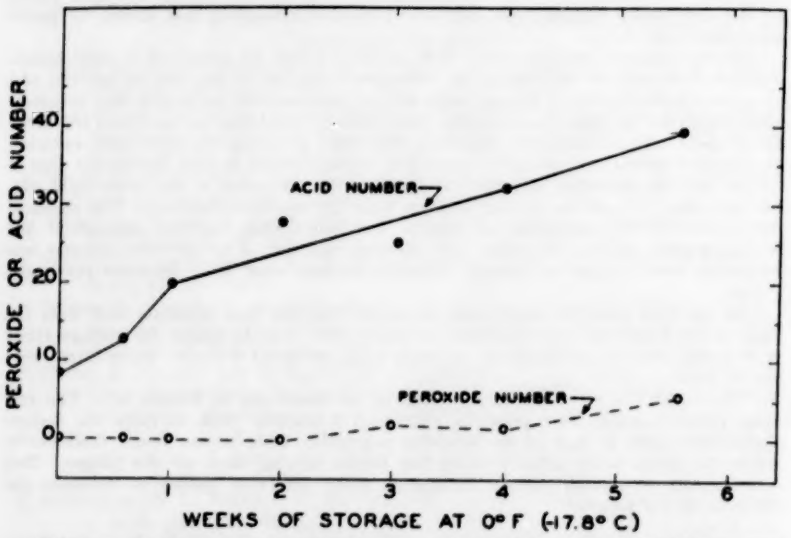


Figure 1. Increase in acid number and peroxide number of crude lipid from raw Thomas Laxton peas.

1954 Samples

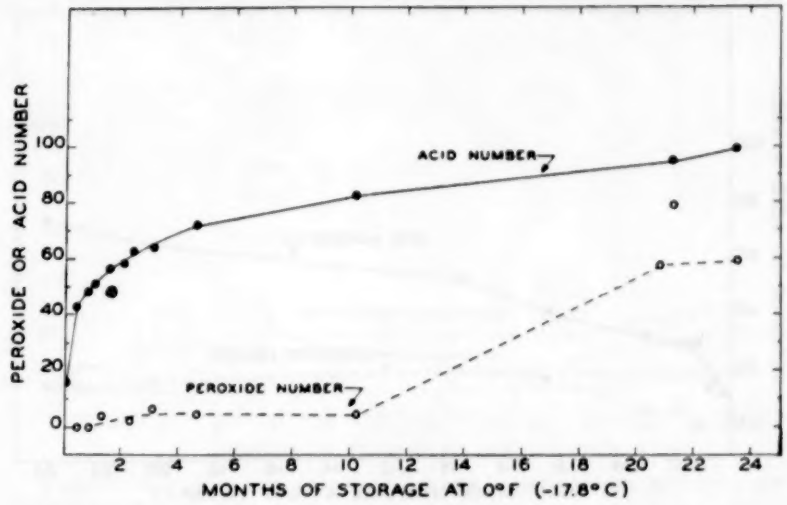


Figure 2. Increase in acid number and peroxide number of crude lipid from raw Thomas Laxton peas.

1952 Samples

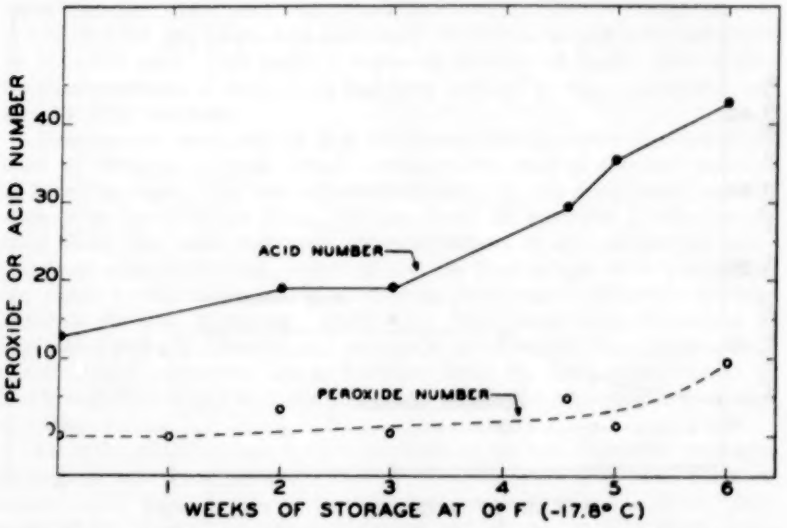


Figure 3. Increase in acid number and peroxide number of crude lipid from raw Wade snap beans.

1954 Samples

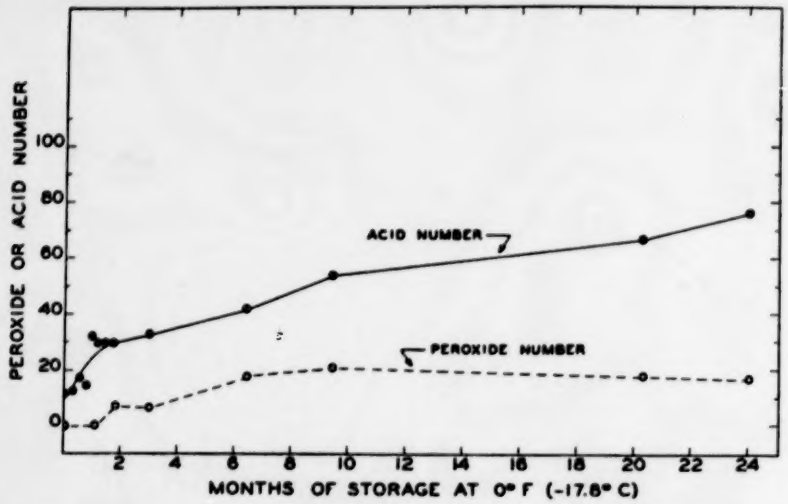


Figure 4. Increase in acid number and peroxide number of crude lipid from raw Wade snap beans.

1952 Samples

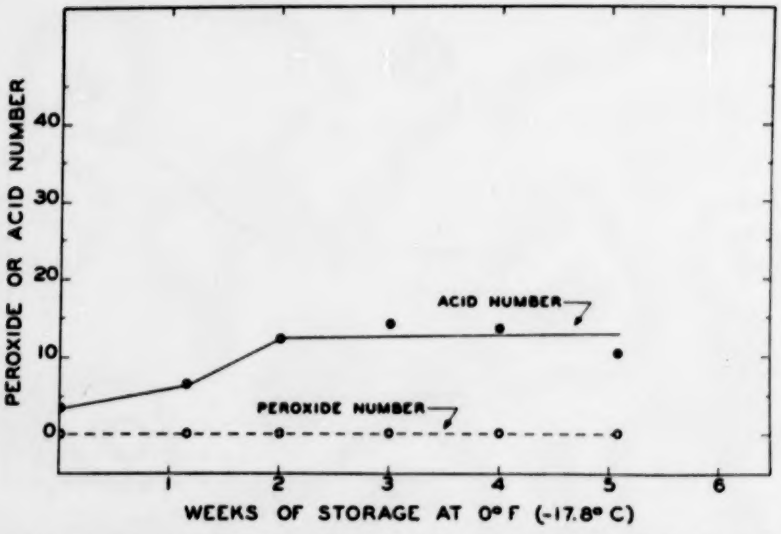


Figure 5. Increase in acid number and peroxide number of crude lipid from raw Golden Cross corn.

1954 Samples

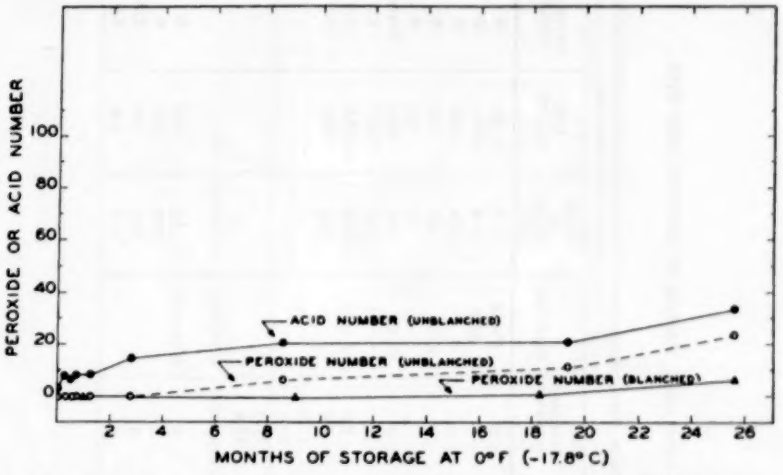


Figure 6. Increase in acid number and peroxide number of crude lipid from raw Golden Cross corn.

1952 Samples

samples only after the acid had already increased significantly. The present results show that the main increase in acid occurred during the first months of storage.

The previously reported *(4)* presence of peroxides in the lipid extracted from blanched sweet corn which had been held in frozen storage at 0°F. (–17.8°C.) for 1½ years, was confirmed. This is in accord with taste tests on blanched corn. This product begins to develop off-flavors after a year in frozen storage, a fact which has been noticed in this laboratory, and also by other workers.

Organoleptic tests showed that the discriminating taster can detect the start of off-flavor in peas within 2 weeks after placing the raw material in frozen storage. This was substantially true for the snap beans and the sweet corn used in this study. It was found in selecting people for the taste panel, that some could not detect off-flavors in the unblanched materials even after fairly long periods of storage. Such people were disqualified for panel membership. This inability to detect such off-flavors perhaps accounts for the statements which have been made that blanching of vegetables prior to freezing and storage is unnecessary. Our results, both chemical and subjective, are in harmony with the taste panel results of Noble and Winter *(6)* that adequate blanching before freezing and storage is indispensable.

Since the off-flavors increased in intensity as the raw vegetables remained in storage and since peroxides started to accumulate later than acids, it seemed likely that a sequence of enzyme actions was involved in the development of the more disagreeable off-flavors and off-aromas (the latter especially noted during cooking) of the unblanched vegetables which had been held in storage for long periods of time. The development of acids suggests

TABLE 1

The progressive development of acidity and peroxide in peas, snap beans, and sweet corn. Analysis of extracted crude lipid.

1952 harvest

Thomas Laxton peas				Wade snap beans				Golden Cross sweet corn			
Condition	Period of storage days	Acid number	Peroxide number	Condition	Period of storage days	Acid number	Peroxide number	Condition	Period of storage days	Acid number	Peroxide number
Raw	16	16.4	0	Fresh	0	11.5	0	Fresh	0	3.0	0
	27	43.1	0	Raw	6	12.2	0	Raw	8	7.4	0
	51	48.9	0		13	17.9	.....		14	7.0	0
	58	50.5	.....		20	14.6	.....		21	8.0	0
	65	45.4	.....		27	32.5	.....		43	8.1	0
	72	56.4	.....		34	29.3	0		86	14.3	Trace
	86	58.8	.....		42	29.4	.....		260	20.7	6.8
	100	62.9	2.7		55	29.2	7.0		586	20.5	11.0
	122	64.5	6.1		90	33.4	6.6		772	32.8	23.2
	168	72.4	3.9		194	41.8	17.9				
	337	82.4	4.9		284	54.7	20.8				
	661	.....	57.3		615	67.5	17.4				
	675	95.1	79.7		731	76.0	16.4				
	742	99.1	59.8								
Blanched	22	11.1	0	Blanched	11	6.5	0	Blanched	16	2.3	0
	656	5.7	0		82	6.7	0		274	3.5	0
					612	10.6	0		554	4.6	1.0
					724	11.2	0		775	4.4	6.0

TABLE 2

The progressive development of acidity and peroxide in peas, snap beans, and sweet corn. Analysis of extracted crude lipid.

1954 harvest

Thomas Laxton peas				Wade snap beans				Golden Cross sweet corn			
Condition	Period of storage days	Acid number	Peroxide number	Condition	Period of storage days	Acid number	Peroxide number	Condition	Period of storage days	Acid number	Peroxide number
Fresh	0	8.5	0	Fresh	0	12.8	0	Fresh	0	3.5	0
Raw	4	12.7	0	Raw	7	.....	0	Raw	8	6.4	0
	7	19.7	0		14	18.7	3.5		14	12.3	0
	14	27.6	0		21	19.1	0		21	14.4	0
	21	25.0	1.7		32	29.5	4.7		28	13.6	0
	28	32.0	1.6		35	35.8	1.1		37	10.7	0
	39	39.3	5.6		42	42.8	9.6				
Blanched	11	7.0	0	Blanched	56	18.6	0	Blanched	41	4.4	0

TABLE 3

Triangle sensory evaluation tests for peas, snap beans, and sweet corn 1952 and 1954 harvests

Year of harvest	Period of storage days	No. of tasters	Average scores			Level of significance
			Fresh	Frozen		
				Raw	Blanched	
Thomas Laxton peas						
1952	21	9		4.1	7.1	0.1
	42	8		4.5	8.2	0.1
	166	12		3.8	7.5	0.1
	873	11		1.4	7.2	0.1
1954	0	9	6.6			
	8	11		6.3	6.6	*
	10	10		5.9	6.5	0.1
	19	9		5.2	7.2	0.1
	26	9		5.2	6.4	0.1
Wade snap beans						
1952	10	8		5.8	7.5	1.0
	24	10		5.3	7.0	1.0
	46	14		5.6	7.0	0.1
	128	9		4.2	7.7	0.1
	834	8		1.6	6.9	0.1
1954	0	9	7.5			
	9	9		5.7	6.8	0.1
	15	10		5.3	6.6	0.1
	23	8		5.0	7.2	5.0
Golden Cross sweet corn						
1952	14	12		5.6	7.2	0.1
	98	9		3.5	8.1	0.1
	702	9		2.0	6.9	0.1
1954	0	9	7.3			
	8	11		6.4	6.9	5.0
	14	10		6.2	7.0	*
	21	10		6.3	7.1	1.0
	28	10		6.6	7.1	5.0
	36	9		5.2	7.5	1.0

* Not significant.

the presence of an active lipase, while the development of peroxides is supposedly the result of lipoxidase activity.

For reasons at present obscure, the rates of development and the amounts of acids and peroxides formed were somewhat different in the 3 vegetables. This may have been due to the amount and activity of the enzymes and substrates present in each vegetable. It seems likely that lipoxidase is related to the more disagreeable off-flavors and off-aromas of the unblanched vegetables held in frozen storage for rather long periods of time. It seems reasonable to assume that the milder off-flavors which developed early in the storage life of the unblanched vegetables were brought about, at least partially, by the increase in acid.

SUMMARY

Unblanched peas, corn, and snap beans showed definite development of off-flavor in frozen storage, which could be detected by a taste panel in from two to four weeks of storage.

The crude lipid matter extracted from frozen unblanched vegetables showed a definite increase in acid after the vegetables were stored for one week. The rise continued during long storage, but the main increase took place during the early months of storage.

The crude lipid matter extracted from frozen unblanched vegetables showed a positive test for peroxides after the vegetables had been held in frozen storage for periods of time as follows: peas, three weeks; snap beans, one month; and sweet corn, two months.

The presence of peroxides in the crude lipid matter extracted from blanched sweet corn after the vegetable was held in frozen storage for 1½ years, was confirmed.

LITERATURE CITED

1. Bengtsson, K., and Helm, E. Principles of Taste Testing. *Wallerstein Lab. Commun.*, 9, 171-80 (1946).
2. Fenton, Faith. The cooking of frozen foods: their nutritive value. *Cornell Bulletin for Homemakers.* Number 628. (December, 1943).
3. Helm, E., and Trolle, B. Selection of a taste panel. *Wallerstein Lab. Commun.*, 9, 181-94 (1946).
4. Lee, Frank A. Chemical changes taking place in the crude lipids during the storage of frozen raw vegetables. *Food Research*, 19, 515-20 (1954).
5. Lee, Frank A., and Wagenknecht, A. C. On the development of off-flavor during the storage of frozen raw peas. *Food Research*, 16, 239-44 (1951).
6. Noble, I., and Winter, J. D. Is blanching necessary when vegetables are to be kept in frozen storage a month or less? *J. Home Econ.*, 44, 33-5 (1952).
7. Roessler, E. B., Warren, J., and Guymon, J. F. Significance in triangular taste tests. *Food Research*, 13, 503-5 (1948).
8. Wagenknecht, A. C., Lee, Frank A., and Boyle, Frank P. The loss of chlorophyll in green peas during frozen storage and analysis. *Food Research*, 17, 343-50 (1952).

NOTES AND LETTERS

DESCRIPTIVE TERMS AND GRADING SYSTEMS

In the preceding issue of FOOD RESEARCH, Sheppard *(7)* refers to my earlier criticism *(1)* of Plank's "Rational Method of Grading Food Quality" *(5, 6)*. In brief, I raised a query as to the value of certain "fundamental suppositions" of Plank's, such as that "it is reasonable to assume that the difference in quality between *good* and *fair* is equal to that between *fair* and *poor*." My criticism was that none of the terms—*quality, good, fair, poor, equal*, and so on—had been properly defined.

To illustrate the results of the ensuing vagueness we can turn to Sheppard's note, where he refers to a certain investigation in which different judges varied a great deal in their interpretation of the grading terms *good, very good*, and others. Now the relevant point here is not whether the difference between *good* and *fair* could still be supposed to be equal to that between *fair* and *poor*, even though people differ in what they mean by *good, fair, poor*, and like terms; instead, the point to be made is that we have no idea whether or not Plank, or Sheppard, or anyone else, would regard this particular experimental finding as relevant to Plank's "fundamental supposition."

The Question of Linearity. Quite a different matter was however raised for Sheppard when I queried Plank's approach, namely, "the whole question of whether scales based on equal sensory intervals can be regarded as linear." This question, which to me is hardly meaningful, is apparently thought to have two aspects, and these must be briefly examined here. (See also *2*).

The first aspect concerns the validity of attaching numerical scores, with their implication of "equal" intervals, to descriptive terms. Consider, then, a set of descriptive terms, for example, *good, fair, and poor*. To these terms we can attach the numerical scores 1, 2 and 3, or the scores 1, 4 and 9, or any scores we like. Which, if any, of these scoring-systems would be valid?

If the scores 1, 2 and 3 are used, the difference between *good* and *fair* will, of course, be *numerically* equal to that between *fair* and *poor*. But to suppose that this implies "equality" in any other sense is to go beyond the stated facts. Furthermore, to suppose that numerically equal units of measurements in physics, say, are necessarily "equal" in any other sense is to go against the *known* facts. Thus, raise the room temperature by 5 degrees, and then by a further "equal" 5 degrees, and again, and again, and see how you feel; drop Newton's famous apple from rest, and note that in its first second of motion it will fall about 16 ft., in the next "equal" second 78 (equal) ft. and so on; remember the law of "marginal utility" in economics, and Weber's Law in psychophysics.

If, on the other hand, any other than the 1, 2, 3 scale is used, this new one is, of course, simply a logical function of the scale above—in particular, the scores 1, 4, 9 can be written as 1^2, 2^2, 3^2. It follows that all such scales are equally valid (or not), and one scale has to be chosen rather than another merely on account of its greater simplicity in practical use.

We now note that in thus considering the relationship between descriptive terms and scores, the question of linearity did not, in fact, arise. The reason is, of course, that the relation of the scores to the descriptive terms can be neither linear nor non-linear, because the descriptive terms are not quantitative at all.

The second aspect which the question of linearity is thought to have concerns the

relationship between some given numerically scored descriptive terms and some other quantitative variable (such as the "sensory intervals" mentioned by Sheppard). Suppose that the second variable is sufficiently well defined so that readings could actually be obtained, and that we have, in fact, parallel observations for each of the two variables. Then the relation between the two observed sets of readings may, or may not, be linear. This is, of course, just a matter of fact. It is not, as Sheppard and certain other writers would seem to imply, a question of whether a scale can be *regarded* as being linearly related to some other variable, but simply whether it *is* so.

Moreover, this question of whether the empirical relationship between two variables is or is not linear depends on the choice of scale for each, which is, as we have already seen, logically quite arbitrary. Thus, for Newton's apple the relation between distance fallen and time taken is non-linear; whilst that between distance and the *square* of the time, or that between the logarithm of the distance and the logarithm of the time, is linear. Similarly, it may well be that under some specific circumstances the scores 1, 2 and 3 for *good, fair,* and *poor* are non-linearly related to some other variable (even to the "equal sensory intervals" mentioned by Sheppard); but this does not in any way affect the *validity* of the scores used. A typical finding in the sensory field is that, under a certain range of conditions, variations in the flavour of fish (scored on some "arbitrary" score from 0 to 10) are related to variations in the fish muscle content of volatile bases (estimated as mg. N per 100 cc. muscle extract) by the law Flavour + 0.68 log (1 + bases) = 15.0 *(4)*. Here it is the chemical variable, measured in units which are "additive," "equal," and so on, which is behaving non-linearly, but it might equally well have been the sensory variable. The validity of neither method of measurement seems to be at stake.

It seems to follow from all this that there is no "deep" question of linearity or the like to be solved in attaching numerical scores to descriptive terms. Indeed, the only thing that seems to matter is actually to attach some numbers which are easy to handle in the initial context, and then—this is the important point—to use the scale. As more and more investigations are carried out with it, the numerical scores will be found to be so useful—almost essential even—in dealing with extensive data that no more time will be spent on discussing the quasi-philosophical questions of scale-construction. Practical questions of scale-construction, on the other hand, will of course arise from time to time. Thus, it is often found more useful to work in centimetres than in inches. Less trivially perhaps, rather than to work with direct bacterial counts, it is often simpler to work with log-counts. Similarly, the initial scoring system for any sensory variable may later be reformulated; but no new principles are required.

An Alleged Difference in Approach. Towards the end of his paper, Sheppard suggests that a marked difference in approach is implied in my discussion of Plank's article, namely the difference between "consumer" tests, and the "laboratory" or "analytical" kind of sensory test. Plank himself did not distinguish explicitly between consumer and laboratory tests, and I certainly did not intend to intrude such a distinction in my criticism; even now I cannot detect any implications of it in my note, and Sheppard himself of course only refers to some quite different paper *(3)*. [In the specific experiments which he refers to, we explicitly did not concern ourselves with consumer reactions, but with the effects of training; we made it quite clear elsewhere in the paper (*3*, p. 483), that consumer reactions were only a part and not the whole of our problem. Sheppard's criticism that our procedures "may well not be helpful where, as is usual, it is important to predict consumer opinion" can only mean that we were *not* doing what he considers usual.] Now that the distinction between laboratory and consumer tests has however been raised, a comment is necessary.

Sheppard apparently thinks that Plank, "as is usual," was interested in predicting consumer reactions, i.e., in the kind of thing Sheppard

himself seems to be concerned with. But it would seem quite likely from the original context *(5)*, that Plank was concerned with quality-grading of food, rather than with assessing, or predicting, the likes and dislikes of a war-time population of consumers. And, of course, quality-grading tends to be much more like laboratory tests, in that a sample is graded *good* or *bad*, say, not according to the specific grader's ideas on the nature of goodness, but according to some more or less fixed, purely descriptive, standards.

Now for our purposes here it does not matter whether Sheppard's consumer-orientated interpretation of Plank's use of the terms *quality, good, fair, poor,* etc., or the alternative quality-grading interpretation is right. The mere possibility of such a fundamental difference in interpretation of the terms only serves to show once again that there is little of value in Plank's general supposition that "it is reasonable to assume that the difference in quality between *good* and *fair* is equal to that between *fair* and *poor.*"

I am indebted to Mr. Sheppard for showing me his note prior to publication, and for patient discussion which has already removed many misunderstandings.

A. S. C. EHRENBERG
Institute of Psychiatry, Maudsley Hospital
Denmark Hill, London

LITERATURE CITED

1. EHRENBERG, A. S. C. On Plank's rational method of grading food quality. *Food Technol.*, 7, 188 (1953).
2. EHRENBERG, A. S. C. Measurement and mathematics in psychology. *Brit. J. Psychol.*, 46, 20-29 (1955).
3. EHRENBERG, A. S. C., AND SHEWAN, J. M. The objective approach to sensory test of food. *J. Sci. Fd. Agric.*, 10, 482-490, 1953.
4. EHRENBERG, A. S. C., AND SHEWAN, J. M. Volatile bases and sensory quality-factors in iced white fish. *J. Sci. Fd. Agric.*, (1955, in press).
5. PLANK, R. Ueber die Wahl eines Bewertungschemas fuer die Qualitaets-Pruefung von Gefriererzeugnissen. *Voratspflege u. Lebensmittelforsch*, 6, 143 (1943).
6. PLANK, R. A rational method for grading food quality. *Food Technol.*, 2, 241 (1948).
7. SHEPPARD, D. Descriptive terms and points systems for rating food qualities. *Food Research*, 20, 114 (1955).

DIFCO

preferred media
for isolation and differentiation
of

ENTERIC PATHOGENS

SALMONELLA—SHIGELLA

Isolation

Bacto-S S Agar
Bacto-MacConkey Agar
Bacto-Bismuth Sulfite Agar
Bacto-Brilliant Green Agar
Bacto-Selenite Broth
Bacto-Tetrathionate Broth Base

Differentiation

Bacto-Triple Sugar Iron Agar
Bacto-S I M Medium
Bacto-Purple Broth Base
Bacto-Purple Agar Base
Bacto-Urea Broth
Bacto-Urea Agar Base

ENTEROCOCCI

Bacto-Azide Dextrose Broth
Bacto-Azide Blood Agar Base
Bacto-S F Medium
Bacto-Phenylethanol Agar
Bacto-Enterococci Presumptive Broth
Bacto-Enterococci Confirmatory Broth
Bacto-Enterococci Confirmatory Agar

ENDAMOEBA HISTOLYTICA

Bacto-Endamoeba Medium
with Bacto-Horse Serum and Bacto-Rice Powder

THE DIFCO MANUAL, *NINTH EDITION,*
including descriptions of these media and their use, is available on request.

Specify DIFCO — the trade name of the pioneers in the research and development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES
DETROIT 1, MICHIGAN